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Phosphorus Nuclear Magnetic Resonance Studies on the Lipid-Containing Bacteriophage PM2†

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ABSTRACT: ^{31}P NMR spectra of intact bacteriophage PM2 virus were obtained in the presence of 60% sucrose. The spectrum is composed of two major components. One is a powder pattern typical of an axially symmetrical motion, and this was assigned to the phospholipid bilayer of PM2 by comparison with the spectrum of the extracted lipid from PM2 and the known structure of this virus. The chemical shift anisotropy was about −47 ppm at 6 °C. The other component was much broader. This was assigned to the packaged DNA of PM2 by comparison with the ^{31}P NMR spectrum of bacteriophage T4, a virus which has no lipids. A powder pattern spectrum of the PM2 nucleocapsid was also obtained in the presence of 6 M urea and 50% sucrose. The spectrum was quite similar to that of PM2. This fact clearly shows that the nucleocapsid still contains a phospholipid bilayer, in contradiction to an earlier model, and that the structural arrangement of the lipid bilayer and DNA in the nucleocapsid is similar to that in the intact PM2. In the temperature-shift experiments, a spectral change of PM2 was observed in the region from 15 to 22 °C for both the phospholipid and DNA components and above 34 °C for the phospholipid component. The temperature dependence of the spectrum of the extracted bulk phospholipid was found to be different from that of the virus. Furthermore, the effect of temperature on the infectivity of this virus in an early stage of the growth cycle was examined. After exposure to a given temperature for 5 min at the start of infection, the yield increased in a stepwise fashion in the range from 15 to 22 °C and had a maximum at 22.5 °C, suggesting a correlation between the viral yield and a structural change of the virion.

Lipid-containing viruses are suitable models for the investigation of the structure and function of biomembranes because of their simple composition. Bacteriophage PM2 (PM2)[1] is one of the best candidates for such studies. PM2 grows on the marine bacterium *Alteromonas espejiana*. Biochemical and physicochemical studies on it have been thoroughly carried out (Franklin, 1974, 1977). The shape of the virus is spherical, and it has a lipid bilayer. The major phospholipids are phosphatidylglycerol (PG), ~60%, and phosphatidylethanolamine (PE), ~40%. PG is located predominantly in the outer leaflet of the viral lipid bilayer, whereas PE predominates in the inner leaflet (Schäfer et al., 1974). The virus contains only four proteins, namely, proteins I, II, III, and IV. Protein I forms small spikes at the vertexes of the outer shell, which is composed of protein II (Hinnen et al., 1974; Schäfer et al., 1974). Protein IV interacts specifically with DNA (Marcoli et al., 1979). The DNA of the virus is a closed supertwisted double helix. In the presence of 4–6 M urea, the nucleocapsid was isolated and was reported to be a lipid-free complex of DNA, protein III, protein IV, and 10% protein II (Schäfer et al., 1978). Recently a detailed model of the structure of PM2 was proposed on the basis of neutron-scattering experiments (Schneider et al., 1978).

In spite of these studies, relationships between the structure and the biological function are not yet clear. In this respect, nuclear magnetic resonance (NMR) offers an attractive possibility, because the energetic perturbation of the sample is small enough to keep it intact and no additional probe molecule needs to be incorporated. Attempts have been made to look at the membranes of some intact viruses or cells. High-resolution ^{13}C NMR of ^{13}C-enriched vescular stomatitis virus (VSV) was measured (Stoffel & Bister, 1975), followed by ^{31}P NMR of VSV (Moore et al., 1977). ^{1}H NMR of chick embryo cells in the absence and presence of myxoviruses or RNA tumor viruses suggested a change in the cell membrane induced by the virus (Nicolau et al., 1978). Recently ^{2}H NMR of intact *Escherichia coli* incorporated with specifically deuterated fatty acids was reported (Gally et al., 1979). There was a good qualitative agreement in the order parameter profile of hydrocarbon chains in the intact membrane and in artificial phospholipid bilayers, indicating the promising future of this method. It can be said from these studies that in order to get useful information by the use of NMR, a particular molecule of a particular system should be labeled for an unambiguous assignment of the spectrum. Furthermore, since the sensitivity of the NMR method is generally very low, a large amount of pure sample is necessary for the measurements. We have chosen to work with PM2 because the virus

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[1] Abbreviations used: ^{31}P NMR, phosphorus nuclear magnetic resonance; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PM2, bacteriophage PM2; $NaDodSO_4$, sodium dodecyl sulfate; buffer A, 1.0 M NaCl, 0.02 M Tris, 0.01 M $CaCl_2$, and 0.1% β-mercaptoethanol, pH 7.5 at 20 °C; buffer B, 0.1 M Tris, 85 mM NaCl, 2 mM NH_4Cl, and 1 mM $MgCl_2$, pH 7.5 at 20 °C.

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is easily purified in large quantity. The most convenient nucleus for our purpose is phosphorus (^{31}P). ^{31}P is located only in the phospholipid bilayer and DNA of the virus, and the natural abundance is 100%. By reducing the rotational motion of the virus, we have succeeded in obtaining a complete powder pattern spectrum of the intact virus. From this spectrum we obtain information not only about the phospholipid bilayer but also about the packaged DNA. Our investigation shows clear evidence for the existence of a lipid bilayer in the PM2 nucleocapsid, in contradiction to the earlier model. Further, we have observed a correlation between a structural change in the virus and some early event in the infectious cycle.

Materials and Methods

Preparation of Samples for NMR Measurements. Highly purified bacteriophage PM2 was prepared according to the procedure reported (Hinnen et al., 1974). When a specific absorbance was used to determine particle number (Camerini-Otero & Franklin, 1975), 20–40% of the particles were infectious. In order to reduce the rotational motion of the virus and to get a high concentration by volume reduction, the virus preparation in buffer A (1.0 M NaCl, 0.02 M Tris, 0.01 M $CaCl_2$, and 0.1% β-mercaptoethanol, pH 7.5 at 20 °C) was subjected to stepwise dialysis against 10%, 30%, and 60% sucrose in buffer A. The largest amount used for one measurement was about 200 mg of virus/0.85 mL. A further advantage of the sucrose-containing buffer was an increase in virus stability measured by the plaque test, as compared to virus in buffer A alone.

The nucleocapsid was isolated as follows: To a given volume of the virus preparation (~15 mg/mL in buffer A) was added dropwise two volumes of the same buffer with 9 M urea at 4 °C with gentle stirring. The mixture was immediately layered on top of a linear gradient (10–30%) of sucrose in buffer A with 6 M urea. After centrifugation at 25 000 rpm (Beckman Type 30 rotor) for 4 h, the nucleocapsid fraction was collected. The ratio of the optical densities at 260 and 280 nm of this fraction was about 1.69. In order to get a high concentration of the nucleocapsid, the nucleocapsid fraction was diluted with the same buffer without sucrose (buffer A with 6 M urea) and was concentrated again on a cushion of 50% sucrose in the same buffer by centrifugation at 30 000 rpm for 6 h. The preparation was characterized by NaDodSO$_4$–acrylamide gel electrophoresis. Phosphorus was analyzed according to Bartlett (1959), protein according to Lowry et al. (1951), and DNA by optical density at 260 nm. The details of the analyses are given elsewhere (Satake et al., 1980).

Purification of bacteriophage T4 was carried out by centrifugation on gradients of CsCl at 25 000 rpm for 6 h (type 30 rotor) (Kellenberger, 1968). The virus fraction was dialyzed against buffer B (0.1 M Tris, 85 mM NaCl, 2 mM NH_4Cl, and 1 mM $MgCl_2$, pH 7.5 at 20 °C) and subsequently dialyzed stepwise against 30% and 60% sucrose in buffer B.

Lipids were extracted from PM2 or its host cell according to Bligh & Dyer (1959). Because the crude extract from PM2 still contained protein IV, it was further purified by silicic acid column chromatography. PE and PG were also obtained from lipid extracts of host cells by silicic acid column chromatography. The purity was checked by thin-layer chromatography. In order to remove divalent cations completely, PE and PG were washed with 10 mM EDTA (~60 mg of PE or PG per 30 mL). Lipid vesicles were dispersed in buffer A with a vortex mixer. The phospholipid pellet obtained by centrifugation was used for NMR measurements.

The phosphorus-31 magnetic resonance spectra at 36.43 MHz were obtained with a Bruker HX-90-FT spectrometer equipped with a home-built quadrature detection system and a variable-temperature unit. Continuous broad-band (0.8 kHz) proton decoupling at 10 W was carried out during the measurements. The temperature unit was calibrated with a standard thermometer; 4096 data points were used with a spectral width of 10 or 20 kHz. A 90° pulse was 12 μs in this system. The delay time adopted was 100 μs, which is longer than the dead time of the receiver. The free induction decays were obtained by using a 4-μs pulse, and more than 40 000 were accumulated for the measurements of virus. The repetition time was 210 ms for 10-kHz and 200 ms for 20-kHz spectral width. Electron paramagentic resonance spectra were recorded at 9.3 GHz on a Varian E-9 spectrometer equipped with a temperature-control accessory. The spin-label Tempo (2,2,6,6-tetramethylpiperidinyl-1-oxy) was used.

Pulse Temperature Shifts in an Early Stage of Infection. PM2 was adsorbed at 0 °C to freshly cultured log phase host bacteria (2×10^8 cells/mL). In order to infect all available cells, a multiplicity of infection of 20 was used. The culture was then divided into 20 1-mL aliquots which were incubated at a series of temperatures for 5 min, after which they were transferred to an incubator at 10 °C. At this time 0.2 mL of trypsin (1 mg/mL) was added to each culture to inactivate free virus. After 5 min, 0.2 mL of soybean trypsin inhibitor (1 mg/mL) was added. The cultures were incubated at 10 °C for 7 h, and their optical densities at 610 nm were monitored during the incubation. The lysates were centrifuged at 10 000 rpm for 5 min, and each supernatant was assayed for infectivity by the plaque test.

Sources of Materials. Bacteriophage T4 was generously donated by Dr. F. Arisaka (from this institute). Calf thymus DNA was purchased from Merck. All other reagents were analytical grade and from common sources (Merck, etc.)

Results

Phosphorus NMR of PM2 and Its Assignments. A proton-decoupled phosphorus NMR spectrum of PM2 in solution was obtained as a powder pattern in the presence of 60% sucrose (Figure 1A), but not in its absence. Since the diameter of PM2 is about 600 Å, the rotational motion of the virus is likely to be fast enough to average out the chemical shift anisotropy. Because the infectivity of the virus checked by plaque test does not change, the virus is still intact in the presence of 60% sucrose. Therefore the appearance of the powder pattern cannot be attributed to the aggregation of the virus. Instead, the rotational diffusion constant of the virus in the presence of 60% sucrose can explain it, as shown in the Appendix. We attempted to measure the spectrum of a centrifuged pellet of the virus. Under this condition, however, the virus was not stable enough at higher temperatures for a long-term measurement to be made.

For assignments of the spectrum of the intact virus, multilayer vesicles of the total phospholipids extracted from PM2 were examined in buffer A (Figure 2A). This is a powder pattern spectrum typical for a molecule which is undergoing an axially symmetric motion as expected for a liquid-crystalline bilayer. Such a spectrum is characterized by two chemical shift components, parallel ($\sigma_\parallel$) and perpendicular ($\sigma_\perp$) to the symmetric axis normal to the bilayer. These two components appear at the edges of the spectrum, as shown in Figure 2A (Seelig, 1978). The difference of these components ($\sigma_\parallel - \sigma_\perp$) is the chemical shift anisotropy. In principle, the spectrum is identical for a bilayer and a monolayer. The comparison of the phospholipid spectrum with that of the virus shows that the spectrum of PM2 contains a powder pattern due to a phospholipid bilayer or monolayer. In the light of X-ray and

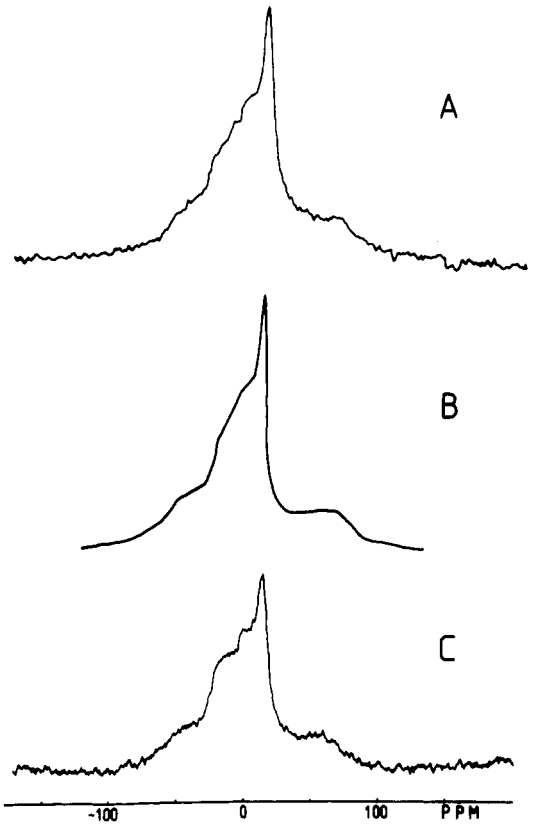

FIGURE 1: ^{31}P NMR spectra of PM2 virus and nucleocapsid. (A) Bacteriophage PM2 in buffer A with 60% sucrose at 6 °C, accumulation of 60 000 free induction decays. (B) Calculated spectrum (see text). (C) Nucleocapsid in 1 M NaCl, 0.1% β-mercaptoethanol, 6 M urea, 50% sucrose, and 20 mM Tris, pH 7.5 at 4 °C; accumulation of 350 000 free induction decays. The repetition time was 200 ms for both. The chemical shift is shown relative to 85% H_3PO_4.

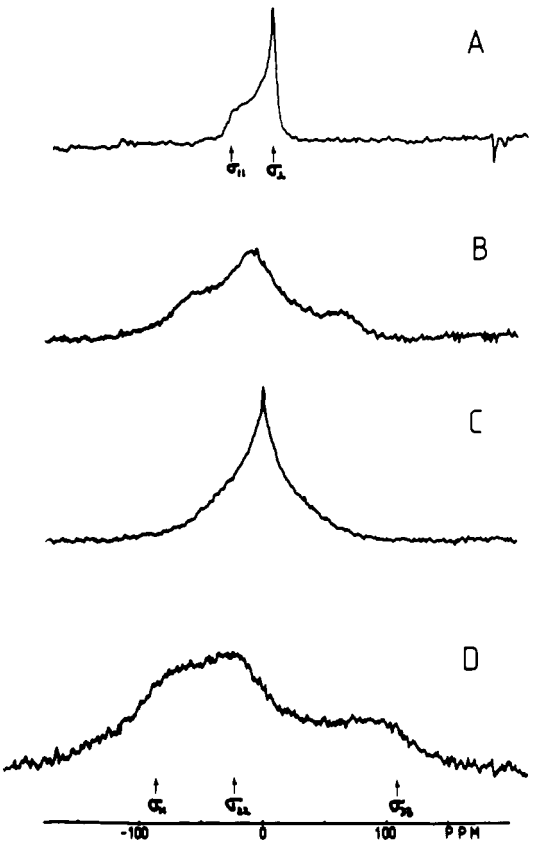

FIGURE 2: ^{31}P NMR spectra of extracted lipids, T4, and calf thymus DNA. (A) Extracted lipids of PM2 in buffer A at 4 °C; accumulation of 20 000 free induction decays. (B) Intact T4 in buffer B with 30% sucrose at 5 °C; accumulation of 60 000 free induction decays. (C) T4 after being heated at 65 °C in buffer B with 30% sucrose at 5 °C; accumulation of 160 000 free induction decays. (D) Solid calf thymus DNA at 21 °C; accumulation of 310 000 free induction decays. The repetition time was 200 ms for all measurements. The chemical shift is shown relative to 85% H_3PO_4. $\sigma_\perp$, $\sigma_\parallel$: chemical shift components perpendicular and parallel to the bilayer normal. σ_{11}, σ_{22}, σ_{33}: the three chemical shift principal elements.

neutron scattering studies (Harrison et al., 1971; Schneider et al., 1978), a monolayer appears to be unlikely. There is another broad component in the spectrum. The contribution of deoxyribonucleic acid (DNA) was checked by using bacteriophage T4, which has no lipid. The phosphorus spectrum of T4 in the presence of 30% sucrose (in buffer B) is presented in Figure 2B. There was no change in the spectrum in the presence of 60% sucrose. This spectrum shows that the broad component of the spectrum of PM2 is mainly due to its DNA. A spectrum calculated from the spectra of extracted lipids and T4 DNA is shown in Figure 1B. The width of the spectrum of T4 DNA was modified to fit the spectrum of PM2. The pattern of the calculated spectrum is quite similar to that of PM2. Therefore the two major components of the spectrum of PM2 can be assigned to the phospholipid bilayer and the DNA.

Rigid Packing of DNA in the Virus. The spectrum of T4 is a typical asymmetric powder pattern (Figure 2B). When DNA was ejected from T4 heads by heating at 65 °C, the structure of the spectrum of the intact virus was lost (Figure 2C). This suggests that the spectrum of the intact T4 is characteristic of the packaged DNA where a segmental motion is strongly hindered. In addition, the change of the spectrum upon release of the DNA could be partly attributed to the change in the hydration of DNA. More hydration could increase the proton–phosphorus dipolar broadening to such an extent that it obscured the asymmetric powder pattern. The phosphorus spectrum of solid calf thymus DNA was measured as a reference for DNA with the most restricted motion. Although the proton decoupling power is not strong enough to eliminate dipolar broadening, the powder pattern is rather typically asymmetric. An asymmetric powder pattern can be characterized by three principal elements of the chemical shift tensor (σ_{11}, σ_{22}, σ_{33}) (Taylor et al., 1975) which are shown in Figure 2D. The principal values of calf thymus DNA are in good agreement with those reported for solid salmon DNA (Terao et al., 1977). In the case of PM2 and T4, $|\sigma_{11}|$, $|\sigma_{22}|$, and $|\sigma_{33}|$ are smaller than those of solid DNA. This means that the chemical shift tensor of phosphorus is partially averaged by the motion of DNA in the virus. Therefore, although the DNA in both T4 and PM2 is packed quite rigidly, it still undergoes a restricted motion. A spectral simulation has been done for T4 by using the formula given (Taylor et al., 1975). The principal elements of the chemical shift tensor obtained from the simulation are −68 (σ_{11}), −9 (σ_{22}), and 87 (σ_{33}) ppm at 4 °C relative to 85% H_3PO_4.

Structure of the Nucleocapsid of PM2. The nucleocapsid of PM2 was reported to be lipid free (Schäfer et al., 1978). Therefore we tried to evaluate the contribution of DNA to the spectrum of PM2 by examining the nucleocapsid. The nucleocapsid was purified in the presence of 6 M urea according to the method reported (Schäfer et al., 1978). Although the concentration of the solution was relatively low (55 mg of nucleocapsid/1.5 mL), a phosphorus spectrum of the nucleocapsid was obtained in the presence of 6 M urea and 50% or 60% sucrose (Figure 1C). The spectrum clearly shows the existence of a phospholipid bilayer. The intensity ratio of the

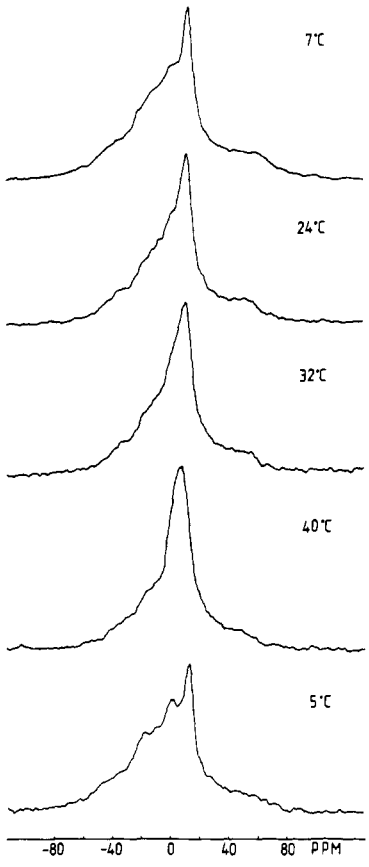

FIGURE 3: Temperature dependence of the ^{31}P NMR spectrum of PM2 in buffer A with 60% sucrose. The bottom spectrum at 5 °C was taken after the sample was brought back down to this temperature from 40 °C. The chemical shift is given relative to 85% H_3PO_4; 40 000 free induction decays were accumulated under the repetition time of 200 ms for each measurement.

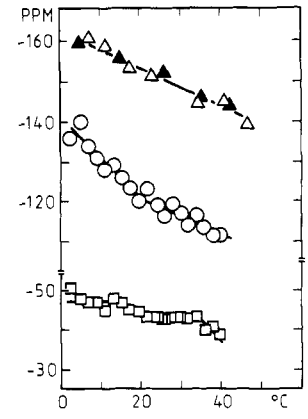

FIGURE 4: $(\sigma_\parallel - \sigma_\perp)$ and $(\sigma_{11} - \sigma_{33})$ of PM2 and T4 viruses as a function of temperature: (□) $(\sigma_\parallel - \sigma_\perp)$ of PM2; (○) $(\sigma_{11} - \sigma_{33})$ of PM2; (▲) $(\sigma_{11} - \sigma_{33})$ of T_4 in the presence of 30% sucrose; (△) $(\sigma_{11} - \sigma_{33})$ of T_4 in the presence of 60% sucrose. $\sigma_\parallel$, $\sigma_\perp$ for the phospholipid component and σ_{11}, σ_{33} for the DNA component were estimated at the half-height of the edges of the spectrum.

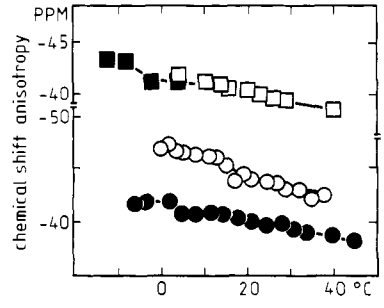

FIGURE 5: Temperature dependences of the chemical shift anisotropies of the extracted phospholipids in buffer A. (□) Bulk phospholipids from PM2 ((■) in the presence of 33% glycerol); (○) phosphatidylethanolamine; (●) phosphatidylglycerol.

phospholipid bilayer component relative to that of the whole spectrum is similar to that of the virus. Therefore most of the phospholipid bilayer in the virus remains in the nucleocapsid provided T_1 values for both samples are similar. The preparation has no protein I and only a trace of protein II in acrylamide gel electrophoresis. Therefore it was not contaminated with the intact virus.

Chemical analysis of the nucleocapsid (Satake et al., 1980) showed that preparations obtained both by Schäfer et al. and by us are identical except for the presence of phospholipid. The spectrum in Figure 1C leads to the conclusion that the nucleocapsid has a lipid bilayer and there is no essential difference in the structural arrangement of DNA and phospholipid between the whole virus and the nucelocapsid. The presence of more than 90% of the original phospholipids in the nucleocapsid was confirmed biochemically, and the presence of the lipid bilayer was suggested by low-angle X-ray spectra as well (Satake et al., 1980). The presence of the lipid bilayer in the nucleocapsid supports the idea that the major protein embedded in the lipid bilayer is protein III (Schneider et al., 1978), since it is the major protein of the nucleocapsid.

Effect of Temperature on the Structure of PM2. Phosphorus NMR spectra of PM2 were measured in approximately 2 °C intervals from 4 to 40 °C, and some typical examples are given in Figure 3. After measurements were taken at several temperatures, aliquots of the sample were taken and infectivity was checked. There was no change in infectivity up to 30 °C, but it was reduced to one-third at 40 °C. From heat-inactivation experiments it is known that the virus is stable up to 35 °C, begins to lose infectivity above 35 °C, and is rapidly inactivated at 50 °C (Tsukagoshi et al., 1975). As can be seen in the bottom spectrum in Figure 3, an isotropic signal due to the damaged virus appears at about 0 ppm after the solution was heated up to 40 °C, while the change of the spectrum was reversible up to 32 °C. The chemical shift anisotropies $(\sigma_\parallel - \sigma_\perp)$ of phosphorus in the phospholipid bilayer and the values $(\sigma_{11} - \sigma_{33})$ of phosphorus in DNA are plotted as a function of temperature (Figure 4). The former parameter contains information on orientation and motion of polar head groups of the phospholipids, and the latter parameter relates to the motion of the DNA. The change in the chemical shift anisotropy of the phospholipid bilayer in intact virus was observed in the regions from 15 to 22 °C and above 34 °C (see Figure 7). $|\sigma_{11} - \sigma_{33}|$ of the DNA decreases continuously with an increase of temperature, and there is an inflection point in the range 18–25 °C. The temperature of the inflection point corresponds to one of the changes in the phospholipid bilayer of the virus. This suggests that a structural change takes place in the viral membrane and the packaged viral DNA simultaneously in this temperature region. Above 34 °C, the chemical shift anisotropy of the spectrum of the phospholipid bilayer begins to decrease significantly, and the spectrum approaches that of an isotropic powder pattern. Since most of the spectrum was reversible on cooling down to 5 °C from 40 °C and one-third of the infectivity remained after this measurement, the spectrum at 40 °C does not represent that of the damaged virus. The significant decrease of the infectivity, however, suggests that the virus is unstable at 40 °C.

For comparison with the intact virus, the temperature dependences of the spectra of extracted phospholipids were ex-

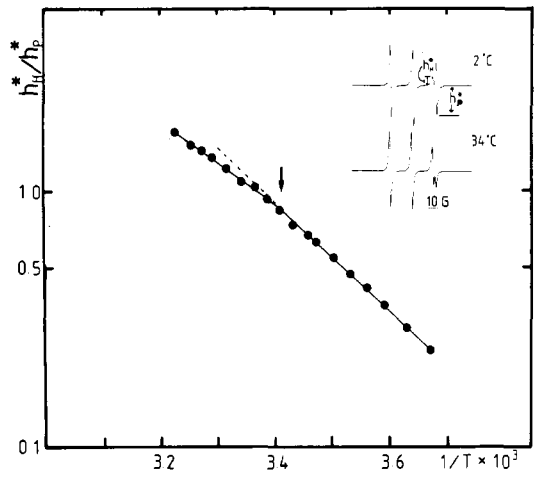

FIGURE 6: Plots of h_H^*/h_P^* of Tempo in phosphatidylethanolamine multilayer vesicles in buffer A ($-\beta$-mercaptoethanol) as a function of $1/T$; ESR spectra at 2 and 34 °C are presented in the insert. The definitions h_H^* and h_P^* are shown in the spectrum. The arrow indicates the inflection point.

amined. All of them showed axially symmetric powder patterns below 50 °C. The chemical shift anisotropies of the extracted mixture of phospholipids in multilayer vesicles, as well as phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) vesicles, are plotted as a function of temperature (Figure 5). PE and PG were isolated from the host cell because it is known that the fatty acid compositions in the virus are identical with those in the host cell (Camerini-Otero & Franklin, 1972). A broad phase transition was observed in the region from −8 to 0 °C and from 13 to 22 °C for the bulk phospholipids and PE, respectively. No detectable transition was observed for PG between −8 and 45 °C. The phase transition of PE was also examined by electron paramagnetic resonance. The intensity ratio of the Tempo signal in the bilayer (h_H^*) to that in the solvent (the same buffer as in the NMR measurement) (h_P^*) was plotted as a function of reciprocal absolute temperature. A discontinuity was observed at about 18 °C (Figure 6), which is in good agreement with the result from NMR. The comparison of the temperature dependence of chemical shift anisotropy between the intact virus and the phospholipids extracted from the virus showed that the arrangement of the lipid bilayer in the virus is different from that in the vesicle. The effect of temperature on the packaged DNA in the absence of a phospholipid bilayer was examined by using T4. The value of $(\sigma_{11} - \sigma_{33})$ of T4 is plotted as a function of temperature in Figure 4. In contrast to PM2, no detectable inflection point was found in the region from 4 to 47 °C. The difference in $\sigma_{11} - \sigma_{33}$ suggests that the packing of DNA in T4 is much more rigid than that in PM2.

Effect of Pulse Temperature Shift in an Early Stage of Infection. In order to see a relationship between the structural change in PM2 and its biological function, the effect of temperature in an early stage of the reproduction cycle of the virus was investigated. After adsorption of the virus to the host cell at 0 °C, the cultures were incubated at 10 °C for 7 h, except during the first 5 min, during which each culture was incubated at a different temperature. Details of the experiment are given under Materials and Methods. The yield of each culture is plotted in Figure 7 as a function of temperature during the first 5 min. The virus production increased in a stepwise fashion in the region from 15 to 20 °C and had a maximum at about 22.5 °C. Lysis started at the same time in each culture. The reciprocal value of the time constant of lysis (the half decay time of optical density at 610 nm) also shows a

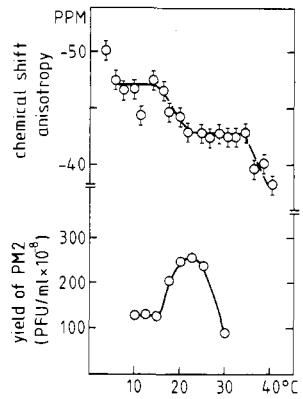

FIGURE 7: Yield of PM2 as a function of temperature during the first 5 min of incubation (O) compared with the chemical shift anisotropy of PM2 (open circle with error bar).

similar curve as in Figure 7 (data not shown). These results suggest that there is a highly temperature-sensitive process in an early stage of infection. The temperature range of the increase of virus production is in good agreement with that of the change of the chemical shift anisotropy of PM2 (Figure 7). That there is a maximum in the pulse shift curve may be due to a second (detrimental) effect which predominates at higher temperatures.

Discussion

Our investigation brings a new insight into the structure of PM2, not only in a static sense but also in a dynamic sense. On the basis of the preceding studies (Schneider et al., 1978) and our results on the nucleocapsid, a model of the virus structure can be proposed. Proteins I and II form spikes and outer shell, respectively. The nucleocapsid is composed of proteins III and IV, a trace of protein II, lipid, and DNA. Protein III is embedded in the lipid bilayer, and the DNA is packaged rigidly inside the envelope of the nucleocapsid. Since nucleocapsid aggregates in the absence of urea, it must have a hydrophobic surface component which can be attributed to protein III. Since protein II forms the outer shell, there must be hydrophobic interactions between proteins II and III. If we look in detail at the shape of the spectrum of the phospholipid bilayer in the nucleocapsid, it is a little bit different from that of the viral bilayer. This suggests the existence of interactions between phospholipid head groups and protein II that could modify the motion and/or orientation of the phospholipid molecules. From biochemical studies it has been suggested that electrostatic interactions between phosphatidylglycerol, a major component of the outer leaflet of the bilayer, and protein II stabilize the virus (Schäfer et al., 1974).

Temperature dependence of the spectrum of PM2 offered an insight into the dynamics of the structure. The shape of the spectrum of the phospholipid bilayer in Figure 3 remains identical up to 15 °C. It begins to change above 17 °C and obviously gets close to an isotropic pattern above 36 °C. This fact means that some motions are averaging the chemical shift tensor. Since the biggest difference of the chemical shift in an axially symmetric powder pattern is $(\sigma_\perp - \sigma_\parallel)$, the characteristic time constant (τ) is the time for a phospholipid molecule to change its orientation between parallel and perpendicular to the magnetic field. The resonances at $\sigma_\parallel$ and $\sigma_\perp$ represent the orientations of the bilayer normal parallel and perpendicular to the magnetic field, respectively. Such motion may be a consequence of rotation of the virus and/or lateral diffusion of the phospholipid molecules in the bilayer. From the estimation of τ (Appendix), it is concluded that only the lateral diffusion of phospholipid molecules in the liquid-

crystalline state can change the shape of the spectrum. Therefore the change of the chemical shift anisotropy in the region from 15 to 22 °C can be assigned to the phase transition which generates the liquid-crystalline state in the higher temperature region. Above 34 °C another change in the viral structure takes place which further facilitates the lateral diffusion of phospholipids.

In the host cell membrane it was shown by using a fatty acid auxotroph mutant that the phase transition temperature of the cell membrane and that of vesicles of the extracted phospholipids are similar (Tsukagoshi et al., 1976). The same has been shown for *E. coli* and *A. laidlawii* membranes (Overath & Träuble, 1973; Steim et al., 1969). In contrast, the phase transition behavior of the phospholipids in PM2 is quite different from that in vesicles. This suggests not only a different arrangement of the lipid bilayer in the virus from that in the vesicle but also a different principle for organizing the membrane of the virus from that of the host cell membrane. Two factors would contribute to this difference, the effect of virus proteins and an asymmetric arrangement of the viral lipid bilayer. A high content of protein III in the lipid bilayer would provide a strong and cooperative protein–protein interaction. Even if phospholipids are not strongly bound, such interaction could impose a cooperative restriction on the motion of the phospholipid molecules which would affect their phase behavior. The reported asymmetric arrangement of the lipid bilayer in the virus (Schäfer et al., 1974) would also introduce such a difference. The similarities in the phase transition temperatures and the chemical shift anisotropies in both the virus and phosphatidylethanolamine vesicles suggest a possibility that the phase transition of the lipid bilayer of the virus could be determined by that of the inner leaflet of the asymmetric bilayer where the major component is phosphatidylethanolamine. So far, however, no direct evidence is available to distinguish between these two factors. Anyway, the change in the envelope structure, including the phase transition of the lipid bilayer, seems to cause a change of the packing of DNA because the appearance of the inflection point (Figure 4) is not an intrinsic property of a packaged DNA in a virus, as we showed here for T4.

In addition, the pulse-temperature-shift experiment implied a correlation between such structural dynamics and biological function. It was concluded in this experiment that an early stage of infection of PM2 is highly temperature sensitive. The temperature dependence of the adsorption rate of PM2 to the host cell reaches a plateau above 15 °C (H. Satake, unpublished results). Therefore the temperature-sensitive process would be attributed to the entry of the virus (or DNA) and/or the initial stage of virus replication. One of the most plausible factors which could control such a temperature-sensitive process is the phase transition of the host cell membrane. It is known, however, that the lipid bilayer of the host cell membrane shows a broad phase transition at about 12 °C (Tsukagoshi et al., 1976) and therefore cannot be evoked to explain the temperature dependence of the virus reproduction. In contrast, the temperature region of the increase of virus reproduction is in good agreement with the phase transition temperature of the viral membrane (Figure 7). It suggests a correlation between the structural change of the virus and the efficiency of infection. This suggestion is supported by the results of a pulse-temperature-shift experiment when elaidic acid or oleic acid enriched virus which were produced by using a fatty acid auxotroph host cell [cf. Tsukagoshi et al. (1975)] was used. The temperature range of the increase of yield was different by a few degrees for these two viruses, although wild-type bacterium was used as host cell for both viruses (H. Satake, unpublished results). In other words the pulse-temperature-shift curve can be attributed to changes in the virion, not in the host cell.

Appendix

Estimation of the Characteristic Time Constant for Changing the Spectral Shape. The rotation of PM2 in buffer and/or the lateral diffusion of phospholipids in the bilayer can change the orientation of a phospholipid molecule in the virus relative to the magnetic field. If the time constant for such reorientation is fast enough, the spectral shape of an axially symmetric powder pattern is modified (Spiess, 1974). When only the rotation of the virus is taken into account, the average time for the reorientation of a phospholipid molecule in PM2 (τ_r) is calculated to be 4.8×10^{-2} s at 5 °C and 9.6×10^{-3} s at 30 °C [$\tau_r = (\pi/2)^2/2D_r$, where D_r = rotational diffusion constant]. The rotational diffusion constant of PM2 in 60% sucrose was calculated by using the hydrated radius (330 Å) and the viscosity of the solvent instead of that of the solution (η). Since τ_r is proportional to η/T where T is the absolute temperature, it changes continuously with temperature. The calculated values clearly show that the rotation of the virus cannot average the chemical shift tensor in the temperature range up to 40 °C.

The lateral diffusion constant (D_l) of the phosphatidylcholine molecule in the liquid-crystalline state is known to be about 10^{-8} cm^2/s (Fahey & Webb, 1978; Rubenstein et al., 1979). The average time to change the orientation of a phospholipid molecule (τ_d) on a sphere with 220-Å radius (Schneider et al., 1978) is calculated to be 6×10^{-4} s in the liquid-crystalline state [$\tau_d = [^1/_4\,(2\pi r)]^2/2D_l$, where r = radius of the sphere]. The lateral diffusion constant of phosphatidylcholine was used in this calculation since that of neither phosphatidylethanolamine nor phosphatidylglycerol is known. It gives $\Delta\omega_{ani}\tau_d \simeq 6$, which is small enough to change the shape of the spectrum (Spiess, 1974). $\Delta\omega_{ani}$ is the absolute value of the chemical shift anisotropy in radians per second. In contrast to virus rotation, a lateral diffusion constant changes its order of magnitude by two through the phase transition (Fahey & Webb, 1978; Rubenstein et al., 1979). Therefore the lateral diffusion does not affect the spectral shape in the gel state at all. This argument accounts for the temperature dependence of the spectral shape very well. Therefore the averaging of the chemical shift of the phospholipids in PM2 can be attributed to the lateral diffusion of the phospholipid molecules in the liquid-crystalline state.

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Excited Indole-3-aldehyde from the Peroxidase-Catalyzed Aerobic Oxidation of Indole-3-acetic Acid. Reaction with and Energy Transfer to Transfer Ribonucleic Acid†

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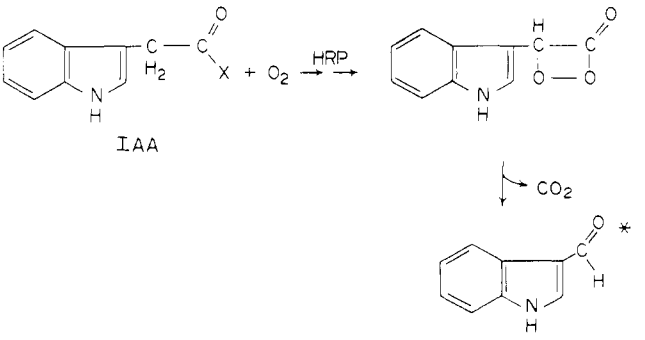
ABSTRACT: The horseradish peroxidase catalyzed aerobic oxidation of the auxin indole-3-acetic acid generates triplet indole-3-aldehyde in high yield. The excited species is quenched by oxygen with formation of singlet oxygen, which is responsible for the observed photon emission and can be trapped by suitable agents. tRNA dramatically enhances the emission as a result of energy transfer from triplet indole-3-aldehyde to a 4-thiouridine group in tRNA. Triplet indole-3-aldehyde also adds covalently to tRNA. The results provide a possible mechanism for the auxin–tRNA interaction in vivo.

The biochemical generation and functionality of electronically excited species—other than those involved in classical bioluminescence—are under investigation in our laboratories (Durán et al., 1977; Faria Oliveira et al., 1978; Cilento et al., 1978; Vidigal-Martinelli et al., 1979; Bechara et al., 1979; Rivas-Suarez et al., 1979; Augusto & Cilento, 1979; Durán et al., 1979; Makita & Durán, 1979; Vidigal et al., 1979; Cilento, 1980; Zinner et al., 1980; Durán & Cilento, 1980; Haun et al., 1980). A system which qualifies for investigation, and is being thoroughly investigated, is the peroxidase-catalyzed aerobic oxidation of IAA[1] to IAl (Vidigal et al., 1975, 1979; Durán et al., 1976; Zinner et al., 1976, 1980). The reaction proceeds, at least formally, through an intermediate dioxetanone (Cilento, 1975); since dioxetanone/dioxetane cleavage affords one of the carbonyl derivatives in an electronically excited state (Kopecky & Munford, 1969; McCapra, 1973; Turro et al., 1973; Bechara et al., 1976; Wilson, 1976; Adam, 1977; Horn et al., 1979), IAl might be generated excited:



IAA IAl

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[1] Abbreviations used: IAA, indole-3-acetic acid; IAl, indole-3-aldehyde; HRP, horseradish peroxidase; HRP-I, horseradish peroxidase compound I; HRP-II, horseradish peroxidase compound II; ISC, intersystem crossing; DABCO, 1,4-diazabicyclo[2.2.2]octane.

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