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A COMPARISON OF THE MOBILITIES AND THERMAL TRANSITIONS OF RETROVIRUS LIPID ENVELOPES AND HOST CELL PLASMA MEMBRANES BY ELECTRON SPIN RESONANCE SPECTROSCOPY

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The lipid bilayers of several type-C retroviruses and selected host cells were spin labeled with 5-doxyl stearic acid, and intact viruses and cells were subjected to electron spin resonance spectroscopy in order to measure lipid mobility. Thermal transition profiles generated for four different retroviruses were dissimilar; differences in the values of the hyperfine splitting constant $2T_{\|}$ and in the positions of thermal break points reflect variations in mobility which can be correlated with the phospholipid/cholesterol molar ratios of the viral envelopes. Moreover, removal of virion surface projections by protease digestion altered the mobility of the envelope and the positions of thermal break points, but the effect observed depended upon the particular retrovirus examined. Studies on retrovirus-infected and uninfected host cells have revealed that persistent virus infection can elicit changes in host plasma membrane mobility and in the positions of thermal break points, the direction and magnitude of which are highly dependent upon the particular retrovirus-host cell system under consideration.

Introduction

Enveloped viruses afford a unique opportunity to evaluate the contributions of lipid and protein to the physical structure of biological membranes. The lipid bilayer which encapsulates certain viruses is, in a sense, a specialized plasma membrane; it is typically comprised of cellular lipid derived as the virus buds from the plasma membrane of its host cell and one or two virus-coded proteins [1]. For example, the envelopes of type-C retroviruses contain surface projections consisting of a hydrophobic 'spike' protein embedded in the lipid bilayer and linked by disulfide bonds to a hydrophilic 'knob' glycoprotein [1–3]. The only other viral protein associated with lipid is a single polypeptide species which forms a continuous mantle beneath the bilayer [1–3]. The simplicity of the viral envelope as compared with the various membranes of a cell recommends its use as a model membrane structure, and yet physical probe analyses of the viral envelope have been rather limited [4–10].

The lack of information appears to be due in part to the difficulty in identifying thermal transitions in virion envelopes. However, we have recently described a procedure which facilitates such an analysis [11], making feasible more detailed studies of virus envelope structure. The lipid bilayers of all enveloped viruses studied to date have been shown to exist as a much more rigid structure than the host plasma membrane [4–11], and while

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it has been suggested that this pronounced rigidity derives from the interaction of viral protein with the bilayer [5], there is little direct evidence as yet to support this contention. As a means of investigating the determinants of viral envelope rigidity, we have subjected several retroviruses to spin label electron spin resonance spectroscopy and correlated rigidity of the viral envelope with its cholesterol content. By removing virion surface proteins through protease treatment, we have been able as well to evaluate the role of protein in determining viral envelope rigidity.

The deposition of retrovirus proteins into the plasma membranes of persistently infected host cells during the virion assembly process can alter a number of membrane characteristics, including antigenicity [1] and lectin affinity [12,13]. However, it would seem that the insertion of virus-specific proteins into the plasma membrane and the concommitant rearrangement of host membrane proteins could also lead to changes in lipid bilayer mobility. In fact, some physical probe studies suggest that enveloped virus infection can alter host plasma membrane mobility [14]. Changes in lipid mobility can result in the modification of membrane receptor activity [12], enzymatic activity [15,16], or membrane transport [17,18], yet little is known about these parameters in virus-infected cells. Thus, we examine here the effect of virus infection on plasma membrane mobility and thermal break point positions in two different retrovirus-host cell systems.

Methods and Materials

Tissue culture. All cell lines were grown in fortified Dulbecco modified Eagle's media supplemented with 5–10% fetal calf serum [2,19]. All cell cultures were removed from bottles or flasks using 0.02% (w/v) EDTA and washed several times in Eagle's medium lacking serum. The washed cells, suspended in medium at a concentration of approx. $20 \cdot 10^6$ cells/ml, were then spin labelled as described below and analyzed immediately.

Virus purification. Friend murine leukemia virus, equine infectious anemia virus and bovine leukemia virus were purified as previously described [2,19]. Avian myeloblastosis virus isolated from the serum of viremic chickens was the generous gift of Dr. Joseph Beard (Life Sciences, Inc., St. Petersberg, FL).

Spin labelling. Procedures employed for spin labelling are based on those described previously [11]. A spin label working solution was prepared which contained 1 mg/ml of 5-doxyl stearic acid (Syva, Palo Alto, CA) plus 50 mg/ml crystallized bovine serum albumin (Sigma, St. Louis, MO) as carrier. 1 mg of the appropriate purified virus suspended in phosphate-buffered saline and 0.2 ml of spin label working solution were mixed and brought to a volume of 1 ml by the addition of phosphate-buffered saline, pH 7.2. The mixture was incubated for 2 h at 25°C, after which unbound spin label was removed by centrifugation of the mixture on a 25–60% (w/v) sucrose gradient for 2 h at 45000 rpm ($240000 \times g$) and 4°C in a Beckman SW 50.1 rotor. The viral band was removed from the side of the centrifuge tube with a syringe and transferred to a quartz aqueous sample ESR flat cell. Under these standard reaction conditions (0.5 mg spin label per mg viral lipid), the final concentration of spin label incorporated into purified virus is less than 0.1% of the virion lipid content, thereby avoiding any detectable perturbation of the envelope structure [4–10,20].

Intact host cells were spin labelled by incubating a minimum of $20 \cdot 10^6$ cells in serum free medium and 0.2 ml of spin label solution, with constant mixing for 2 h at 25°C. Spin-labelled cells were washed several times in medium and pelleted in a clinical centrifuge. The final cell pellet was resuspended in a minimal volume of medium (about 10^8 cells/ml) and transferred to an ESR flat cell. The spin label utilized in these studies, 5-doxyl stearic acid, is known to intercalate into the plasma membrane bilayer such that the long axis of the probe molecule is essentially parallel to the fatty acyl chains of the bilayer phospholipids; there is no evidence that the spin label can penetrate beyond the bilayer of the plasma membrane [4–10,21,22].

ESR spectroscopy. All ESR spectra were obtained on a Varian E-109 ESR spectrometer and analyzed as described by Slosberg and Montelaro [11]. Temperature was regulated to within 0.1°C using a temperature control device of our own design. Temperature measurements were made with a chromel-constantan thermocouple (Omega

Engineering, Stamford, CT) which was attached to the face of the ESR flat cell. Thermal transition data was plotted with the aid of an iterative least squares program utilizing normalized B-splines which was developed especially for the identification and statistical evaluation of thermal transition points [23].

Bromelain digestion. Protocols for the specific and complete removal of surface proteins from retrovirus envelopes have been described in detail [2,24] and were modified only slightly for this study. A bromelain stock solution was prepared by suspending 2.0 g of bromelain powder (pineapple bromelain, Grade II, Sigma, St. Louis, MO) in 5.0 ml of 0.02 M phosphate buffer, pH 7.2, containing 0.15 M NaCl and 0.5 mM *p*-chloromercuriphenylsulfonate. The suspension was centrifuged for 20 min at 10000 rpm ($9000 \times g$) in a Sorvall SS34 rotor, and the resulting supernatant was used as the stock solution. A standard reaction mixture consisted of 0.4 ml of stock bromelain solution, 1 mg of spin-labelled virus, and 0.2 ml of 0.2 M Tris buffer, pH 7.2, containing 1 mM EDTA and 20 mM 2-mercaptoethanol. After incubation for 3 h at 37°C, the mixture was diluted to 2 ml with 0.01 M Tris-HCl buffer, pH 8.1, containing 0.001 M EDTA and 0.3 M NaCl. This mixture was layered onto a 3 ml 25–60% (w/v) sucrose gradient and centrifuged at 45000 rpm ($240000 \times g$) for 2 h at 4°C in a Beckman SW 50.1 rotor. The viral band was removed from the side of the centrifuge tube with a syringe and used immediately for ESR scanning. In order to evaluate the efficiency of the removal of virion surface proteins, a parallel sample of 1 mg of virus plus 10^6 cpm of [^{3}H]glucosamine-labelled virus, in which only the surface glycoproteins are labelled [3,19], was digested and purified as described above. After centrifugation, the sucrose gradient was fractionated, and the distribution of tritium label on the gradient was determined by liquid scintillation counting. Protease digestion routinely resulted in greater than 95% of the ^{3}H-label remaining at the top of the gradient; less than 5% of the radiolabel, but greater than 90% of the starting viral protein, was localized in a visible viral band at a characteristic density of approx. 1.16 g/ml [2,24]. These results indicate a quantitative removal of the retrovirus surface proteins under the reaction conditions described.

Plasma membrane extraction. Cell plasma membranes were isolated according to the method of Roozemond [25]. Approx. $20 \cdot 10^6$ cells were disrupted with a glass cell homogenizer, and the resulting homogenate centrifuged at $300 \times g$ for 15 min in a clinical centrifuge. The supernatant was then recentrifuged for 20 min at 6500 rpm ($3900 \times g$) in an Sorvall SS34 rotor and the mitochondrial pellet discarded. The remaining supernatant was centrifuged for 1 h at 26000 rpm ($80000 \times g$) in a Beckman SW 50.1 rotor, and the resulting supernatant discarded. The microsomal pellet was resuspended and homogenized in 1 ml of 40% sucrose in 10 mM Tris (pH 7.4) buffer using a tight fitting glass homogenizer. The volume was then adjusted to 2.5 ml with 40% sucrose/10 mM Tris buffer solution and overlaid with 2.5 ml of 30% sucrose/10 mM Tris buffer. The step gradient so produced was centrifuged for 18 h at 26000 rpm ($80000 \times g$) and 4°C in a Beckman SW 50.1 rotor. A single band, corresponding to the enriched plasma membrane fraction, was removed from the side of the gradient with a syringe.

Phospholipid and cholesterol determinations. Samples of intact virus, and isolated host cell plasma membranes were made 0.2 M in NaCl and then extracted with 3 vol. of chloroform/methanol (2:1, v/v). The resulting organic phases containing lipid material were then analyzed for phospholipid content according to the method of Duck-Chong [26] and for cholesterol content by the modification of Johnson [27] of the cholesterol-oxidase method of Richmond [28].

Results

Comparison of the lipid envelopes of four different retroviruses

Fig. 1. illustrates plots of $2T_{\parallel}$ versus temperature obtained from several retroviruses, utilizing the 5-doxyl stearic acid spin label. The spectral parameter $2T_{\parallel}$ was measured from scans as previously described [11]. These type-C retrovirus particles are morphologically very similar [1,3,19] and yet an examination of their thermal transition profiles reveals several interesting differences. The values of the hyperfine splitting constant ($2T_{\parallel}$), which have been shown to correlate with lipid

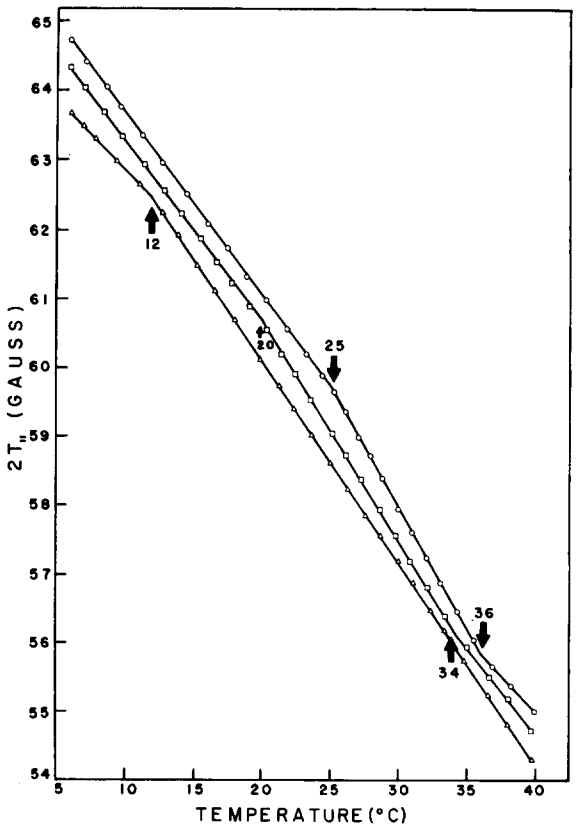

Fig. 1. Plots of $2T_{\parallel}$ versus temperature for 5-doxyl stearic acid spin labelled bovine leukemia virus (○——○), Friend murine leukemia virus (□——□), and equine infectious anemia virus (△——△). The plot for avian myeloblastosis virus is virtually indistinguishable from that of Friend virus and is therefore not shown separately. Arrows indicate the positions of computer localized break points.

bilayer mobility and which are comparable to measures of mobility in other biophysical techniques [29–31], suggest that at all temperatures, including physiological temperature (37°C), the lipid bilayer of bovine leukemia virus is the most rigid (least mobile) while the lipid envelope of equine infectious anemia virus is the least rigid (most mobile) of those depicted in Fig. 1. The thermal transition plots for the lipid bilayers of avian myeloblastosis virus and Friend murine leukemia virus are virtually indistinguishable, and the intermediate position of their transition plot correlates with an intermediate degree of lipid bilayer rigidity. Moreover, the lipid bilayer of bovine leukemia virus displays the highest break temperature (25°C), that of equine infectious anemia virus the lowest break temperature (12°C), while the bilayers of avian myeloblastosis virus and Friend murine leukemia virus display and intermediate break temperature (20°C). In the case of Friend murine leukemia virus a second break occurs at 34°C [11], while bovine leukemia virus displays a second break at 36°C (Fig. 4, Table I). All thermal breaks described in this communication have been reproduced with a precision of ±0.5°C.

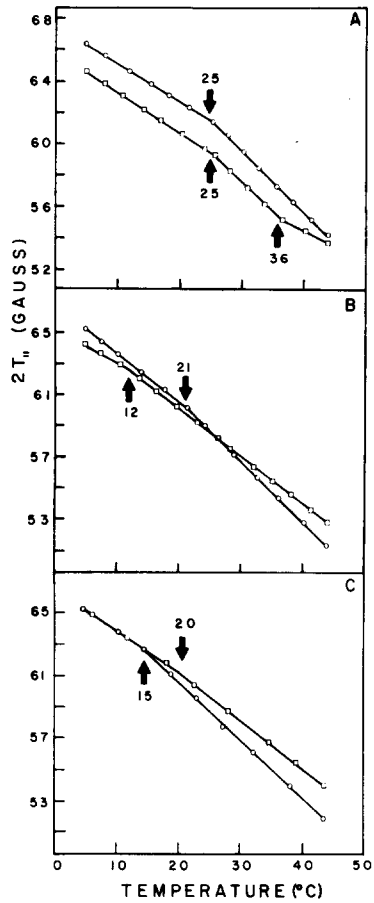

Fig. 2. Effect of bromelain treatment of virus on plots of $2T_{\parallel}$ versus temperature. (A) Untreated bovine leukemia virus (□——□), bovine leukemia virus treated with bromelain (○——○). (B) Untreated equine infectious anemia virus (□——□), equine infectious anemia virus treated with bromelain (○——○). (C) Untreated avian myeloblastosis virus (□——□), avian myeloblastosis virus treated with bromelain (○——○). Arrows indicate the positions of break points.

TABLE I

PHOSPHOLIPID/CHOLESTEROL MOLAR RATIOS AND THERMAL BREAK POINT TEMPERATURES OF SEVERAL RETROVIRUSES AND SELECTED HOST CELLS

Sample		Molar P/C ratio [a]	Break points (°C) [b]		
			Lower	Middle	Higher
Virus samples					
Bovine leukemia		0.55		25	36
Avian myeloblastosis		0.67		20	
Friend murine leukemia		0.64		20	34
Equine infectious anemia		0.82	12		
Uninfected host cells [c]					
Fetal equine kidney		0.88	10	23	35
Fetal lamb kidney		2.72	8		29
Infected host cells [c]					
Cell	Infecting virus				
Fetal equine kidney	Equine infectious anemia	0.84	10	23	35
Fetal lamb kidney	Bovine leukemia	2.80	9		30
Eveline mouse embryo	Friend murine leukemia	0.76	14		29

[a] Molar P/C ratio = molar phospholipid/cholesterol ratio (±0.05).
[b] Thermal break points from plots of $2T_{\parallel}$ versus temperature (±0.5°C).
[c] Data refer to the plasma membrane of the cell.

A strong correlation seems to exist between the phospholipid/cholesterol molar ratio of each viral envelope (Table I) and values of $2T_{\parallel}$ obtained from plots of $2T_{\parallel}$ versus temperature. Studies have shown that in eukaryotic cells this ratio often varies directly with lipid bilayer mobility [32,33], a consequence of the stiffening action of cholesterol. The results described here indicate that retrovirus envelope mobility can also be correlated with phospholipid/cholesterol molar ratios.

Contribution of surface polypeptides to viral envelope rigidity

In order to investigate further the parameters determining viral envelope mobility, retrovirus surface proteins were proteolytically digested, and the effect of this treatment on bilayer mobility examined. Proteolytic digestion with bromelain was estimated to have removed greater than 95% of the viral knob and spike structures based on a greater than 95% loss of viral associated [^{3}H]glucosamine label following proteolysis (as discussed in Methods).

Fig. 2A illustrates that bromelain-treated bovine leukemia virus displays higher values of $2T_{\parallel}$ than untreated virus over the entire temperature range studied. In addition, bromelain treatment removes the 36°C thermal break seen in untreated virus. Thus, the knob and spike structures of bovine leukemia virus appear to lessen the rigidity of the viral bilayer over a broad range of temperatures.

The effect of bromelain digestion on the thermal transition plot of equine infectious anemia virus is rather complex. As shown in Fig. 2B, the bromelain-treated virus is more rigid than untreated virus below 25°C, but more mobile above 25°C; at 37°C (physiological temperature), the digested virus is significantly more mobile than untreated virus. It is also evident that the break temperature is increased from 12°C to 21°C after bromelain treatment. Thus, the effect of equine virus spike and knob structures on bilayer mobility is temperature dependent, although the surface proteins clearly immobilize the bilayer at physiological temperature.

The effect of bromelain treatment of avian

myeloblastosis virus is similar to that observed with equine virus. As shown in Fig. 2C, bromelain-treated and untreated avian virions exhibit identical plots of $2T_{\parallel}$ versus temperature, and thus identical lipid mobilities, below 15°C. Above 15°C the lipid envelope of the treated virus displays progressively smaller values of $2T_{\parallel}$ (and hence greater mobility) than the envelope of the untreated virus. It is also apparent (Fig. 2, Table I) that the 20°C break temperature of intact avian myeloblastosis virus has been shifted downward to 15°C after bromelain treatment. Thus, the spike and knob projections of avian myeloblastosis virus, like those of the equine virus, induce a decrease in envelope lipid mobility at physiological temperature.

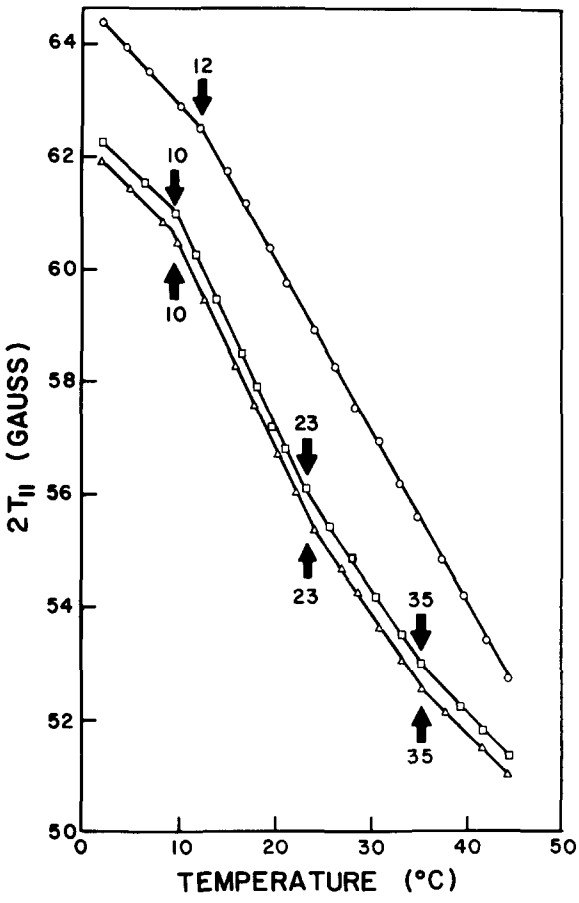

Fig. 3. Plots of $2T_{\parallel}$ versus temperature for equine infectious anemia virus (○——○), uninfected fetal equine kidney cells (□——□), and infected fetal equine kidney cells (△——△). Arrows indicate the positions of break points.

Effect of virus infection on host plasma membrane mobility

Of the four type-C retroviruses studied, bovine leukemia virus and equine infectious anemia virus represented two for which both virus-infected and uninfected host cells were available. We therefore conducted a series of experiments designed to examine the effect of persistent virus infection on host plasma membrane mobility.

In one such experiment, fetal equine kidney cells were infected with equine infectious anemia virus, and aliquots of infected cells, uninfected cells, and intact virus were isolated and spin labelled as described in Methods and Materials. Samples were subjected to ESR spectroscopy and plots of $2T_{\parallel}$ versus temperature were generated. As shown in Fig. 3, the slight downward displacement of the infected cell temperature plot as compared with the uninfected cell plot suggests that the infected cells have slightly more mobile plasma membranes. With regard to thermal break points, however, there is no difference between infected and uninfected cells; three breaks can be observed, which occur at 10, 23, and 35°C. The phospholipid/cholesterol molar ratios of the plasma membranes of infected and uninfected fetal equine kidney cells (Table I) are identical within experimental error (0.84 and 0.88, respectively), a finding which is consistent with the similarity of the infected and uninfected cell plots. Also evident from Fig. 3 is the pronounced rigidity of the equine infectious anemia viral envelope relative to the host plasma membrane, as described for other enveloped viruses [4–11]. Since the phospholipid/cholesterol molar ratios of virus and cell are identical within experimental error (Table I), this rigidity does not appear to derive from the stiffening action of cholesterol.

In a similar experiment, fetal lamb kidney cells were infected with bovine leukemia virus; aliquots of virus, infected cells, and uninfected cells were spin labelled, and plots of $2T_{\parallel}$ versus temperature generated. Fig. 4 illustrates the results of this experiment. At every temperature examined, the higher values of $2T_{\parallel}$ corresponding to the plasma membranes of infected cells suggest that these membranes are less mobile than the plasma membranes of uninfected cells. As can be seen in Fig. 4, the two break points associated with the unin-

fected fetal lamb kidney cell plasma membrane (8°C and 29°C) are shifted upward by one degree to 9°C and 30°C after the cell is infected with bovine leukemia virus. This one degree change was reproducible in duplicate studies, but represents the smallest increment over which a statistically significant change might occur. The phospholipid/cholesterol molar ratios of the plasma membranes of infected and uninfected fetal lamb kidney cells are the same within experimental error (Table I). This finding suggest that the observed decrease in membrane mobility accompanying virus infection can not be explained by an increase in the cholesterol concentration of the host plasma membrane, but must be due instead to alterations in membrane protein composition and/or organization. The data in Fig. 4 also demonstrate that the lipid envelope of bovine leukemia virus is more rigid than the host cell plasma membrane from which it is derived. Determinations of the phospholipid/cholesterol molar ratio of bovine leukemia virus consistently result in a value of 0.55, which is 5-times lower than the host cell value of 2.80 (Table I). Thus the rigidity of the bovine leukemia viral envelope may in part be due to the stiffening action of cholesterol.

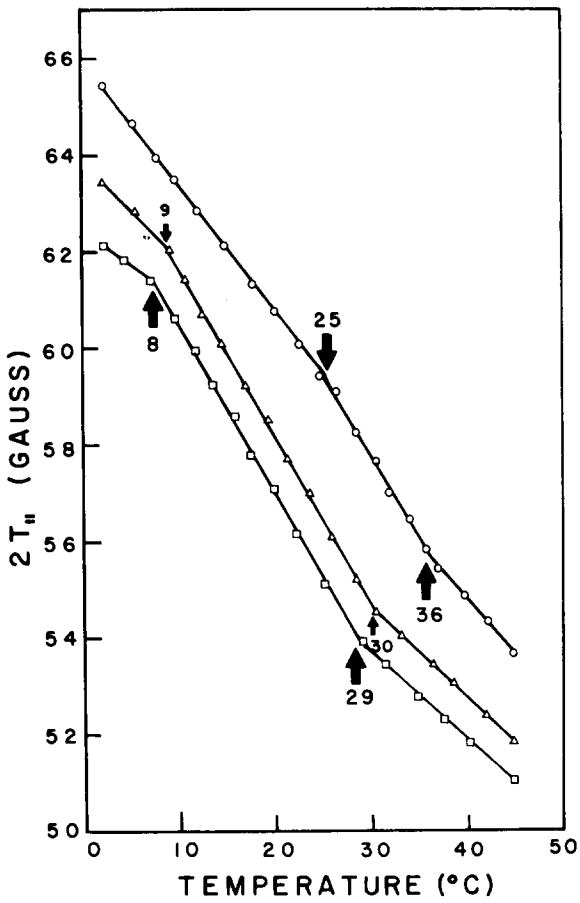

Fig. 4. Plots of $2T_{\parallel}$ versus temperature for bovine leukemia virus (○——○), uninfected fetal lamb kidney cells (□——□), and infected fetal lamb kidney cells (△——△). Arrows indicate the positions of break points.

The reproducibility of plots of $2T_{\parallel}$ versus temperature and of the positions of thermal break points was evaluated with respect to the age of host cells and the homogeneity of successive viral isolates. Aliquots of intact cells and virus were isolated and spin labelled at one week intervals over a period of at least four weeks post-infection with virus. During this period of time, variations in plots of $2T_{\parallel}$ versus temperature and in the positions of thermal break points did not exceed ± 0.5°C. Thus, aging of cells in tissue culture and differences between successive virus isolates could be ruled out as possible explanations for the various virus-induced changes described above.

Discussion

The phenomenon of viral envelope rigidity has been addressed by several investigators who have examined the lipid bilayers of vesicular stomatitis virus, Sindbis virus, and influenza virus [4–7,10,40]. The retrovirus thermal transition plots illustrated in Figure 1 agree with these studies in that they describe viral envelopes which are rigid compared with their host plasma membranes, but the retrovirus envelopes are distinct from each other with respect to degree of envelope rigidity and positions of thermal break points. These differences among retrovirus envelopes appear to correlate with their characteristic phospholipid/cholesterol molar ratios (Table I).

In our studies with bovine leukemia virus and the host fetal lamb kidney cell, we have revealed that the relative cholesterol content of the bovine leukemia viral envelope is five times that of its host plasma membrane (Table I). This increased concentration of cholesterol in the bovine leukemia virus lipid bilayer apparently contributes to the

rigidity of the viral envelope compared to its host plasma membrane. Other workers have reported that the cholesterol content of enveloped virions can exceed by as much as 2-fold that of the host plasma membrane [35–39]. Presumably, the increased cholesterol level in some virions derives from specific interactions between viral protein and cellular lipid during the assembly process, resulting in a specific, rather than random, accumulation of cellular lipid within the viral envelope. The results of the bromelain experiments in which removal of surface knob and spike proteins actually increased the lipid rigidity relative to whole virus (Fig. 2A) suggest that the bovine leukemia virus surface proteins have no significant role in restricting lipid mobility.

Variations in cholesterol content, however, do not seem adequate in every case to explain the pronounced rigidity of the viral envelope as compared with the host plasma membrane. In the case of equine infectious anemia virus and its host fetal equine kidney cell, the phospholipid/cholesterol molar ratios are nearly identical (0.82 and 0.84, respectively), and yet the envelope of the virus is significantly more rigid than the plasma membrane of the host (Fig. 3). Likewise, the lipid bilayer of Friend murine leukemia virus is significantly more rigid than the plasma membrane of its host, the Eveline line of mouse embryo cells [11], and yet molar phospholipid/cholesterol ratios are comparable (0.64 and 0.76, respectively, Table I). Hence, it is probable that the increased rigidity of the murine and equine viral envelopes relative to their host plasma membranes can not be attributed to differences in cholesterol content. Differences in the chain length and unsaturation of the fatty acids which comprise phospholipids and sphingolipids can not be excluded as possible determinants of viral envelope rigidity, but seem unlikely candidates to account for this phenomenon. Studies by a variety of investigators [35,40,41] have established that in virtually every instance the fatty acid composition of the host plasma membrane is identical to that of the viral envelope.

In viruses, such as equine infectious anemia and Friend leukemia, where lipid composition clearly cannot account for enhanced envelope rigidity, it is logical to inquire whether the virion envelope-associated proteins might restrict lipid mobility. The protease experiments described here suggest that proteins interacting with the viral bilayer can indeed restrict lipid mobility, but do so with a complex temperature dependence. The basis of this temperature dependence is difficult to explain, but it is evident that at physiological temperatures (37°C), the presence of knob and spike proteins in intact equine and avian viruses results in a more rigid envelope than is found when the viruses lack these envelope components. Thus these results provide direct evidence that virus envelope-associated proteins can contribute to the rigidity of the viral envelope.

Other investigators have reported that proteolytic digestion of the surface projections of influenza virus [4,7] and Sendai virus [5] had no effect on bilayer mobility, whereas in the cases of Sindbis virus [34,42] and vesicular stomatitis virus [4], a minor increase in envelope rigidity was observed. It is important to note, however, that some of these studies where conducted at 25°C only and, thus, did not examine the effect of protease treatment on viral envelope mobility at other temperatures, including physiological temperatures. An examination of Figs. 2B and 2C reveals that differences detected at 37°C are greatly diminished or absent at 25°C. Hence, evaluations of the contribution of envelope proteins to lipid rigidity must be examined over a range of temperatures, or at least physiological conditions.

A second effect observed after bromelain digestion of equine and avian viruses is a shift in thermal break point temperatures (Fig. 2). By analogy to studies [43–50] of cell plasma membranes in which slope changes in a plot of $2T_{\parallel}$ versus temperature have been ascribed to a gel-liquid crystalline phase transition, the thermal breaks observed in viral bilayers may represent the onset and completion of a viral phase transition. Although cholesterol tends to diminish the gel-liquid crystalline phase transition [46,47], we feel that the subtle but reproducible breaks observed with viral bilayers are suggestive of a similar thermotropic phenomena in the viral envelope. The protease experiments demonstrate that envelope proteins also influence thermal break temperatures, but the effect varied between the retroviruses examined, precluding any generalization about the protein-lipid interactions.

A comparison of the plots of $2T_{\parallel}$ versus temperature in Figs. 3 and 4 reveals that virus infection elicits a change in host cell plasma membrane mobility, the direction and magnitude of which is highly dependent upon the particular virus and host cell under consideration. In the case of fetal equine kidney cells infected with equine infectious anemia virus, there is a slight increase in plasma membrane mobility (lower values of $2T_{\parallel}$) associated with virus infection, while bovine virus infection evidently decreases the mobility of the host fetal lamb kidney cells. Increased membrane mobilities have been reported with influenza virus infection of avian erythrocytes [51] and arbovirus infection of host cells [52]; decreased membrane mobility was observed after infection of baby hamster kidney cells with vesicular stomatitis virus [14]. The significance of these latter results are difficult to evaluate in that the virus-host cell systems employed result in relatively rapid cell death following virus infection. Hence membrane alterations may only reflect a general deterioration of the infected cell. In contrast, the retrovirus system described here is a persistent virus infection leading to stable, inherited alterations in membrane properties. This characterisitic provides a distinct advantage as a model for studying the functional significance of virus-induced membrane alterations, which may be of critical importance in understanding how certain cellular functions in the virus-infected cell differ from those in the uninfected cell.

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