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The fusion peptide of simian immunodeficiency virus and the phase behaviour of *N*-methylated dioleoylphosphatidylethanolamine

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Abstract

Temperature-scan X-ray scattering was used to study the effect of the fusion peptide of simian immunodeficiency virus (SIV) on the lipid polymorphism of *N*-methylated dioleoylphosphatidylethanolamine (DOPE-Me), in the presence and absence of one or both of the fusion inhibitors carbobenzoxy-D-phenylalanine-L-phenylalanine-glycine and 1-lauroyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC). Using X-ray diffraction at stations 2.1 and 8.2 of the Synchrotron Radiation Source at Daresbury Laboratory, UK, the structure of multilamellar vesicles (MLVs) was probed as the temperature was raised from 20 to 90 °C. The results are compared to those of similar studies, reported earlier, that used the fusion peptide of feline leukaemia virus (FeLV) which, at 28 amino acid residues in length, is considerably longer than the SIV peptide (12 amino acid residues). We interpret the results within the framework of current understanding of membrane fusion, and demonstrate how observed lipid polymorphism might describe the fusion process.

Keywords: Small angle X-ray diffraction; Biomembrane fusion; Inhibitor; Lamellar phase; Cubic phase; Hexagonal phase

1. Introduction

The fusion of phospholipid bilayers is an integral part of a number of biological processes, including mitosis, exocytosis, spermatozoid–egg fusion and viral infection [1]. However, the molecular rearrangements of the lipids and the precise kinetic events involved are still uncertain. This is largely because the fusion event is transient and involves only local, isolated patches of lipid. It is clear that biomembrane fusion is a protein-regulated event [2,3]. In order to introduce their infective nuclear material into a host cell, enveloped virus particles use membrane fusion, catalysed by specialised, extra-membranous glycoprotein 'spike', of which the best known is hemagglutinin of the influenza A virus. Viral fusion proteins usually contain a highly conserved N-terminal region that has been shown to be crucial to the process of fusion between the viral envelope and a membrane of the host cell during the infection process. Short peptides (10 to 30 residues long) of corresponding sequence, termed *fusion peptides*, retain much of the membrane fusion activity of the larger protein, albeit with slower rates and lack of a specific binding function. They have enabled research on membrane fusion mechanisms to focus on the fundamental interactions between peptide and phospholipid, in isolation from membrane proteins, and their added levels of complexity.

The precise molecular events that occur during peptide-induced membrane fusion are still unclear. The membrane

Abbreviations: CBZ-D-FFG, carbobenzoxy-D-phenylalanine-L-phenylalanine-glycine; d_H, lattice repeat distance (hexagonal phase phospholipid); d_L, lattice repeat distance (lamellar phase phospholipid); d_Q, lattice repeat distance (cubic phase phospholipid); DOPC, dioleoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; DOPE-Me, *N*-methylated dioleoylphosphatidylethanolamine; EDTA, ethylenediaminetetra-acetic acid; FeLV, feline leukaemia virus; H, hexagonal phase; L, lamellar phase; LPC, 1-lauroyl-2-hydroxy-*sn*-glycero-3-phosphocholine; MLV, multilamellar vesicle; p15EK, fusion peptide from spike protein p15E of FeLV; PIPES1,4-piperazinediethanesulfonic acid; Q, cubic phase; q, $2\pi/d$; SAXS, small angle X-ray scattering; SIV, simian immunodeficiency virus; T_H, temperature at which hexagonal phase is first observed; T_Q, temperature at which cubic phase is first observed

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leaflets, composed of phospholipid molecules, must rearrange into highly curved intermediates prior to fusion pore development [4,5]. These intermediates can also be induced by fusion peptides. It has been demonstrated that peptides that lower the bilayer (L_α)-to-inverted hexagonal (H_{II}) phase-transition temperature in model membranes can also promote membrane fusion through this kind of bilayer destabilization [6]. It is recognised that a large fraction of the lipids of many biological membranes form the H_{II} phase when purified and hydrated under physiological conditions [7]. These lipids have been termed *nonbilayer lipids* in contrast to the *bilayer lipids* that form lamellar structures under the same conditions. It has been suggested that, although membranes are bilayers most of the time, the high concentration of nonbilayer lipids has functional significance in allowing the membranes to form transient nonbilayer structures because vital processes, including fusion, would be topologically impossible with intact bilayers.

A concept that has proved very useful in the attempts to understand fusion peptide activity has been *monolayer curvature strain*. Indeed, it has been proposed that this property is homeostatically controlled in living biomembranes [8]. Current models of fusion peptide function are usually based upon their effect on the monolayer curvature strain of the membranes involved in the fusion process. The peptides are thought to destabilise lamellar bilayers by increasing the hydrophobic volume of a monolayer, relative to the volume of the solvated polar groups [9].

Highly curved lipid mesomorphs, similar to those involved in the fusion process, recently were directly observed for the first time with diphytanoyl-phosphocholine lipids, however, under non-physiological, dehydrated conditions [10]. Similar structures are suspected to occur during the L_α-to-Q_{II} (inverted cubic) phase transition and the L_α-to-H_{II} phase transition [11]. The ability of a number of agents to promote fusion appears to be correlated to their ability to lower the L_α-to-H_{II}-transition temperature (*T*H) [12–14]. Similarly, some fusion inhibitors raise T_H [15]. Although the Q_{II} and the H_{II} phases, which are kinetically stable, are unlikely to exist at the site of a developing fusion pore, knowledge about the topology of the interface as these phases begin to form has clear implications for our understanding of biological fusion mechanisms.

This paper describes a continuation of the work previously reported [16,17] in which we have used temperature-scan X-ray scattering to study the effect of the fusion peptide of feline leukaemia virus (FeLV) on the lipid polymorphism of *N*-methylated dioleoylphosphatidylethanolamine (DOPE-Me). In the more recent paper, we have reported how the tri-peptide carbobenzoxy-D-phenylalanine-L-phenylalanine-glycine (CBZ-D-FFG) and the lipid 1-lauroyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC) modify the phase transition of this lipid. Here we extend the studies to a different fusion peptide, that from simian immunodeficiency virus (SIV), and discuss the findings for this peptide in the context of the model of membrane fusion presented in the earlier paper [17]. The work also related to a study of SIV peptide on phospholipid phase transitions by Colotto et al. [18], who collected X-ray diffraction data at a range of constant temperatures, ranging from 15 to 80 °C.

2. Materials and methods

2.1. Sample preparation

The fusion peptide from the GP160 protein of SIV (Macaque isolate, SWISSPROT accession number P05885, sequence GVFVLGFLGFLA), and the fusion peptide p15EK of FeLV (strain C/Sarma, SWISSPROT accession number P06752, sequence EPISLTVALMLGGLTVGGIAAGVGTGTK), were synthesised and purified by Albachem (Edinburgh, Scotland). CBZ-D-FFG was obtained from Sigma (St. Louis, MO). LPC and DOPE-Me were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification.

Multilamellar vesicles (MLVs) were prepared by rehydrating phospholipid films as previously described [16,17]. The buffer used for rehydration was 20 mM PIPES, 1 mM EDTA, 150 mM sodium chloride and 0.3 mM sodium azide at pH 7.4. All samples had a low lipid concentration of 100 mM, equivalent to 7.57% (w/v), to ensure an excess water condition. The lipid dispersions were then subjected to five freeze–thaw cycles. Repeated freezing and thawing across the chain-melt temperature ensures that the lipid is fully hydrated regardless of the thermal history of the lipid.

2.2. Temperature-resolved X-ray diffraction

The X-ray diffraction experiments were performed at stations 2.1 and 8.2 of the Synchrotron Radiation Source at Daresbury Laboratory, UK. The experimental set-up at the two instruments was essentially identical. The X-ray wavelength was 0.154 nm. The specimen-to-detector length was approximately 1.5 m. Each sample was contained in a glass capillary tube, held in a steel block with electronic (Linkam) programmable temperature control. A thermocouple fixed to the sample chamber monitored the temperature continuously and wrote the temperature into the data files. The temperature increased in a linear fashion at a rate of 30 K/h, a rate originally chosen because the metastable cubic phase of DOPE-Me is not normally observed. Each frame of data collection lasted for 30 s. The effect of thermal radiation from X-ray beams of this kind was minimal. Aluminium foils were added to attenuate the incident beam until no loss of diffracted intensity was observed throughout the duration of a complete temperature scan.

2.3. Data analysis

The program XOTOKO [19] was used to correct for sample thickness and variations in detector response, and to

subtract the background counts. Detector response was determined by measuring a fixed source, ^{59}Fe, overnight both before and after data collection. Calibration of the *x*-axis for small angle scattering was achieved by using rat-tail collagen as a standard [20]. This calibration was repeated prior to the exposure of each new sample. The location, width and amplitude of each Bragg peak were determined by fitting Lorentzian distributions (PeakFit, SPSS Ltd.).

3. Results and discussion

Fig. 1 shows the relationship between lattice basis vector length and temperature for all observed phases of DOPE-Me with respect to temperature. Fig. 2 is a schematic summary of the phase behaviour of DOPE-Me in the presence of SIV fusion peptide and/or fusion inhibitors, as a function of temperature. Relevant data from our earlier papers [16,17] are reproduced for comparison.

3.1. SIV and FeLV

In earlier papers [16,17], we have discussed the effects of the fusion peptide from feline leukaemia virus (FeLV); with the current data, we are now able to compare the two peptides in terms of their effects upon the phase behaviour of DOPE-Me. Each of the peptides is thought to be helical in its active form [21] and Brasseur has proposed that both insert at an oblique angle into phospholipid bilayers. To date, this has only been determined experimentally for SIV [22], though a molecular dynamics study of a related fusion peptide from human immunodeficiency virus also showed oblique insertion [23]. The most striking difference between the two peptides used in this study is their length. FeLV peptide, with 28 amino acids, is considerably longer than SIV peptide, with only 12. In α-helical conformation, this corresponds to a length of 42 Å for FeLV peptide and 18 Å for SIV. Clearly, FeLV peptide may span an entire bilayer, even at a tilt, while SIV reaches across just one leaflet. This may explain why FeLV fusion peptide is always more potent than SIV, in terms of its effects on the phase behaviour of DOPE-Me.

3.1.1. Lamellar phase

Both peptides reduce the temperature of breakdown for the lamellar phase, SIV by 5 °C and FeLV by more than 10 °C. This may be a direct consequence of the peptides' length. Whereas SIV is easily incorporated in two populations in DOPC bilayers [22] and its influence is directed more towards individual monolayers, the hydrophobic length of FeLV requires a full bilayer to span, thus its influence on bilayer breakdown is more direct.

3.1.2. Hexagonal phase

The hexagonal phase is the dominant high-temperature phase for DOPE-Me both with and without FeLV. The lamellar to hexagonal transition is narrow, and in pure DOPE-Me there is a brief coexistence region. SIV, on the other hand, induces a gap between the phases where no structure is present. This brief dissolving of the lipids is perhaps expected, if we consider that SIV acts on individual leaflets, rather than the entire bilayer, making it easier to form micellization products. An important difference between the actions of the two peptides is that while FeLV peptide lowers T_H (by 6 °C), the SIV peptide raises T_H (by 10 °C). The hexagonal phase repeating structural motif is essentially a bilayer. Although FeLV partitions more easily in such a phase than actual lamellar bilayers, the direct influence of SIV on monolayers means that it is readily incorporated into non-bilayer phases, as we shall see. At 2% SIV, the hexagonal phase is eliminated altogether. However, since inverse hexagonal phase is not observed in samples cooled back to 20 °C without a fusion peptide present, it appears that SIV peptide does indeed stabilise this phase, in keeping with our previous observation that inverse hexagonal phase is closely related to the fusion process. The importance of the lamellar to hexagonal pathway in fusion will be discussed in a later section.

3.1.3. Cubic phase

Both FeLV and SIV have similar effects of lowering T_Q, SIV by 10 °C, and FeLV by as much as 20 °C. With 1% SIV, the cubic phase comes to dominate the high T regions, eventually coexisting with a lesser hexagonal phase. The cubic phase with FeLV and 2% SIV is only transient; however, the SIV-induced cubic phase is shifted 15 °C to higher T. In addition, with FeLV the cubic gives way to the hexagonal phase but with SIV the lipid loses all structure. In all cases, the cubic phase is metastable; this phase was observed in samples that had been allowed to cool back to room temperature. The only means to return the sample to pure bilayer form is to incubate the sample for at least 1 h well below the main transition of the lipid.

In terms of its effect upon T_H and T_Q, 2% SIV peptide is clearly not as potent as 1% SIV. This could be caused by aggregation of the hydrophobic peptide, as we have previously speculated for FeLV peptide [24], or it could be that the phospholipid monolayer curvature strain becomes "saturated" between 1% and 2% SIV peptide. Support for the latter comes from similar measurements in the presence of fusion inhibitors, where the 2% peptide produces a lower onset temperature than 1% peptide for the H_{II} phase in the presence of either LPC or CBZ-D-FFG. In addition, the Q_{II} phase that eventually forms in the presence of 2% SIV peptide has a smaller d_Q, and therefore a tighter Gaussian curvature, than that which forms with lower concentrations of the peptide.

3.2. CBZ-D-FFG and LPC

In our previous work on FeLV, the fusion inhibitors had a clear and pronounced opposite effect to that of the peptide itself. Both CBZ-D-FFG and LPC reversed the H_{II} promot-

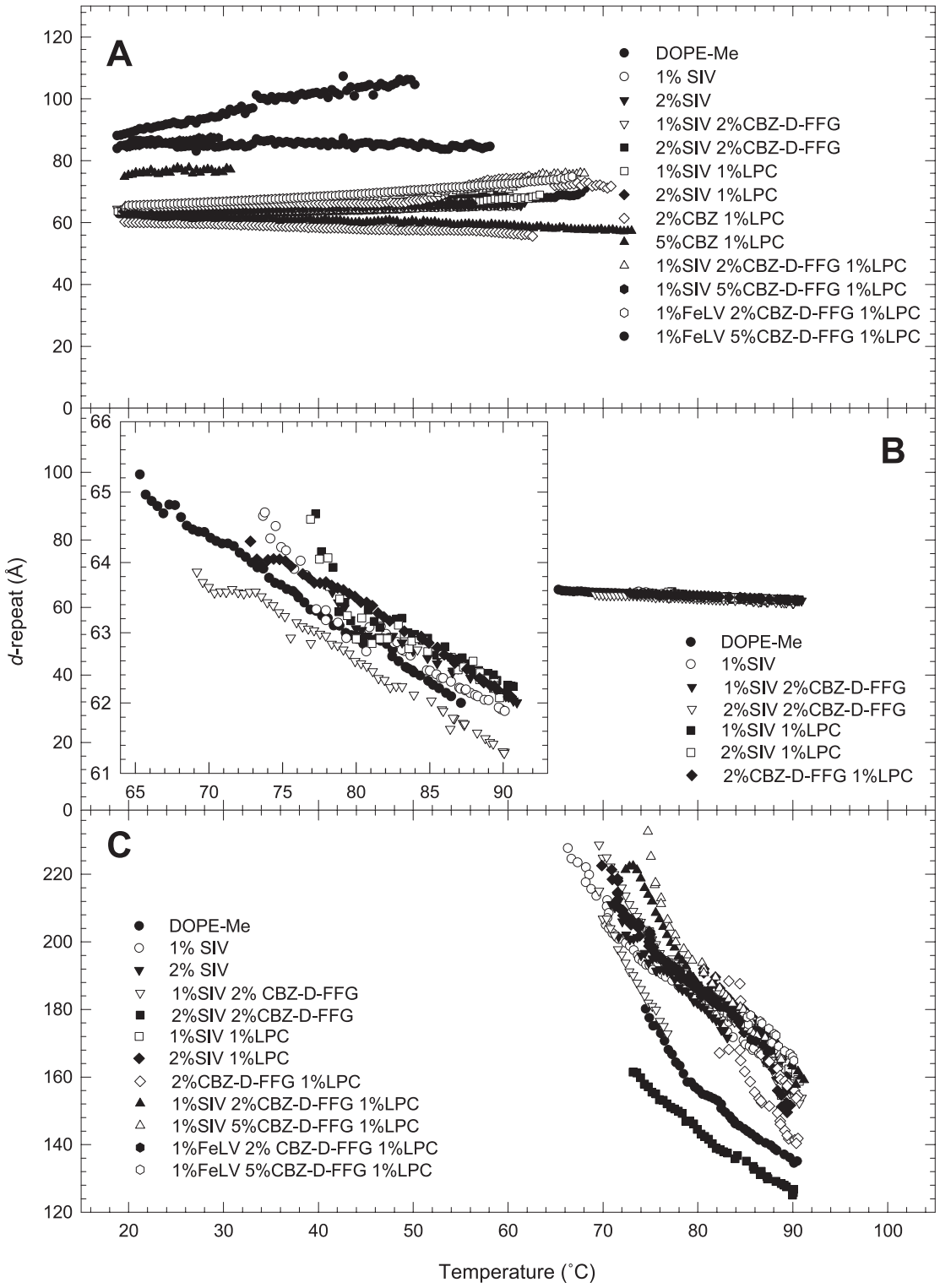

Fig. 1. The relationship of the *d*-repeat (Å) to temperature (°C) for all observed phases of DOPE-Me as determined by temperature-scan X-ray scattering at stations 2.1 and 8.2 of the Synchrotron Radiation Source at Daresbury Laboratory. (A) Lamellar; (B) inverse hexagonal (H_{II}); (C) inverse cubic (Q_{II}). For ease of comparison, the vertical scale is the same for all three panels, though displaced by 120 Å in panel C. Only alternate data points are shown, for clarity. The inset figure in panel B is an enlarged version of the H_{II} plot.

ing effects of FeLV, and promoted large *d*-spacing cubic phases, and some lamellar stability instead. With SIV, the fusion inhibitors once again counteract the peptide's effects on non-lamellar phases; however, this time, they allow for the presence of a metastable H_{II} phase along with the typical cubic phase.

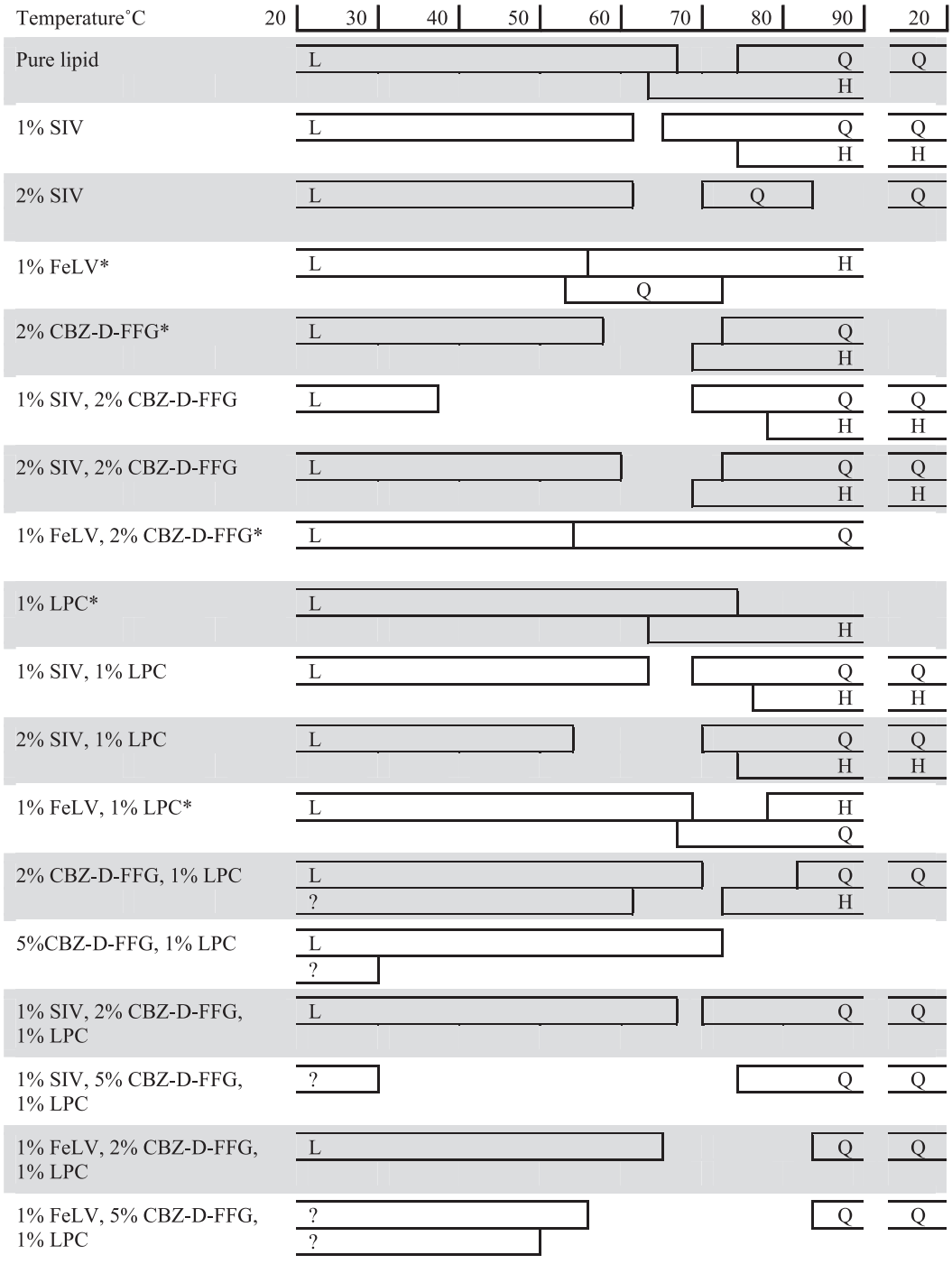

Fig. 2. Schematic summary of the phase behaviour of MLVs of DOPE-Me and DOPE-Me with fusion peptides from SIV and FeLV and/or fusion inhibitors, as a function of temperature. L, lamellar; H, inverse hexagonal; Q, inverse cubic. Data from samples indicated with an asterisk came from a previous study [17].

3.2.1. Lamellar phase

We have previously reported the micellization of the lamellar phase with as little as 2% CBZ-D-FFG. With the similar effect of SIV on the lamellar phase, it is not surprising that their combination dramatically increases the breakdown of that phase. In contrast, the lamellar stabilization properties of LPC are maintained in the presence of SIV. This disparity on the effect on lamellar bilayers between CBZ-D-FFG and LPC is shown dramatically when they are combined. In only the dilute samples of CBZ-D-FFG and LPC were lamellar phases identifiable. In all cases of 5% CBZ-D-FFG with LPC, a single diffraction peak at large *d*-spacing (70 Å) was seen, precluding the identification of the phase. The addition of FeLV or SIV restored the lamellar phase.

Considered from another perspective, the mystery phase seen in the CBZ-D-FFG/LPC combination samples is seen

with the addition of LPC to otherwise protein-solubilised lipids. LPC forms type I (headgroup exposed) tightly curved micelles, thus we might conclude the peptide (SIV and/or CBZ-D-FFG)-dissolved lipids are type II, where the lipid acyl-chain that might be exposed to the water is protected by the peptides. In combination, LPC is structure-stabilizing by offsetting the preference for SIV and CBZ-D-FFG for the hydrocarbon core of a curved monolayer. The large *d*-spacing of the mystery phase may then be explained as a swollen lamellar phase, where the peptides have been sequestered into the centre of the bilayer, increasing its fluctuations, and in turn its overall *d*-spacing.

3.2.2. Hexagonal and cubic phases

Both fusion inhibitors have nearly the same effect on the formation of the hexagonal and cubic phases in the presence of SIV. For 1% SIV, T_H and T_Q are slightly raised by the inhibitor. For 2% SIV the H_{II} phase is restored, and T_H is slightly lower than in 1% SIV. The Q phase is no longer transient, and T_Q is even slightly higher than in 1% SIV. When combined, CBZ-D-FFG and LPC drive the lipids to a Q phase that is highly metastable at elevated temperatures. This Q phase overcomes any effects of the SIV and FeLV peptides, although FeLV is clearly more potent as the onset of the Q phase is delayed by 10 °C. The similarity of effects on the non-lamellar phases, despite the profound differences on the lamellar phase, is significant. The stable non-lamellar phase outwith the normal fusion pathway is a cubic phase with *d*-repeat of 160–220 Å. Hexagonal phases, and presumably hexagonal and fusion precursors, are eliminated entirely.

3.3. The H_{II} phase and fusion pathways

DOPE-Me was chosen for these experiments because of its ability to monitor the effects of fusion agents on monolayer curvature strain. Our previous work with FeLV peptide and fusion inhibitors [17] has told us that there is some feature of the action of fusion peptides that is revealed by their ability to induce inverse hexagonal phases, which is quite separate from their similar ability to induce cubic phases. The lamellar–hexagonal pathway (in DOPE-Me) must share common ground with fusion pathway (in other lipids) *and* this feature must not be present in all lamellar–cubic pathways (there are many cubic phases and there many be many transition pathways). Although membrane curvature-strain clearly plays an important role in fusion, it appears that it is not the only factor involved.

A fluid monolayer is able to respond to a non-zero value of spontaneous curvature by deforming into a curved surface. In this way, the monolayer can accommodate substantial discrepancies between the cross-sectional area per lipid at the headgroup region (A_h) and the corresponding area of the fatty acyl chains (A_c). However, in a bilayer this flexibility is frustrated by the opposing tendencies of the two monolayers, as well as the entropic energy cost of water exposure in the hydrocarbon region. In a planar bilayer, the area per phospholipid molecule is assumed to be constant at all depths of the bilayer ($A_h = A_c$). Increasing temperature will tend to expand the tail region (A_c) more than the headgroups (A_h), due to the increased conformational disorder of the chains, thereby increasing the frustration [25]. This build up of frustration is energetically unfavourable and eventually the system must reduce it by rearrangement of the phospholipids into a curved phase, such as the inverted hexagonal (H_{II}) or inverse cubic (Q_{II}) phases. One way of understanding the effect of fusion peptides and inhibitors upon phospholipid phase transitions is to consider their role in increasing or reducing the frustration within bilayers, as the temperature is steadily increased.

The monolayer curvature strain hypothesis predicts that the addition of agents that affect the curvature strain (such as fusion peptides or inhibitors) should change the Gaussian curvature, at any given temperature, in those phases where such a change is possible. Agents that increase A_c relative to A_h should result in structures with a reduced radius of curvature, and those that increase A_h relative to A_c should show an increase in radius of curvature. One of the most remarkable findings of the present study is how small is the effect of fusion peptides or fusion inhibitors on the lattice repeat of the H_{II} phase in DOPE-Me. In our measurements, the change is only just above the level of experimental error. Colotto et al. [18] have reported that SIV peptide reduces d_Q by a very small amount (<0.25 Å); a mutant, non-fusogenic, analogue of the peptide had no measurable effect at all. Fig. 1 shows that, when plotted on the same vertical scale as the lamellar and cubic data, the repeat distance at any given temperature and, therefore, the radius of curvature of the H_{II} phase remain effectively unchanged, no matter which agents are present. It is only when the vertical scale is exaggerated, as in the inset panel, that small differences in d_Q appear, at a level that is close to the limits of experimental error. The presence or absence of peptides or inhibitors changes the temperature at which the onset of H_{II} phase occurs, but does not change the lattice dimensions. It could be that these agents only affect small, localised regions, and their concentration is too low to have a visible effect on d_Q. If this were true, it would be reasonable to expect to see an increase in the mosaic spread of the H_{II} samples, reflected in the width of the diffraction peaks. However, the FWHM of the first-order Bragg peaks from all H_{II} samples (with/without peptide/inhibitors) is 0.03 ± 0.02 $Å^{-1}$ (q). The peaks do not get broader with peptide or inhibitors, implying that these agents do not disrupt the inverse hexagonal structure, even in localised areas. This may explain why higher concentrations of peptide seem to be less effective in promoting H_{II} phase. It is almost as if the peptides are most effective when they induce earlier phase transitions, without modifying the actual structure formed.

In summary, we have reconfirmed our earlier hypothesis that precursors of membrane fusion are common to the lamellar to inverted hexagonal phase. The mechanism of

bilayer destabilization between the fusion peptides SIV and FeLV differs due to their folded conformational lengths; SIV acts on leaflets of lipids interacting within the hydrocarbon matrix, while FeLV prefers mixing into curved bilayers (such as the H_{II} phase). The net destabilization effect leads to fusion. When CBZ-D-FFG or LPC is added, they tend to undo curvature-strain effects and promote Q phases at higher temperatures, bypassing the intermediate structures that lead to hexagonal formation. These insights should lead us to refine theories of the mechanism of membrane fusion, such as tests for whether lipid mixing between leaflets occurs during the fusion process.

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References

[1] J.M. White, Viral and cellular membrane fusion peptides, Annu. Rev. Physiol. 52 (1990) 675–697.
[2] P.A. Bullough, F.M. Hughson, J.J. Skehel, D.C. Wiley, Structure of influenza hemagglutinin at the pH of membrane-fusion, Nature 371 (1994) 37–43.
[3] F.M. Hughson, Enveloped viruses: a common mode of membrane fusion? Curr. Biol. 7 (1997) R565–R569.
[4] D.P. Siegel, The modified stalk mechanism of lamellar/inverted phase transitions and its implications for membrane fusion, Biophys. J. 76 (1999) 291–313.
[5] A.E. Spruce, The first milliseconds of the pore formed by a fusogenic viral envelope protein during membrane fusion, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 3623–3627.
[6] R.F. Epand, I. Martin, J. Ruysschaert, R.M. Epand, Membrane orientation of the SIV fusion peptide determines its effect on bilayer stability and ability to promote membrane fusion, Biochem. Biophys. Res. Commun. 205 (1994) 1938–1943.
[7] V. Luzzati, in: D. Chapman (Ed.), Biological Membranes, vol. 1, Academic Press, NY, 1968, pp. 71–123.
[8] S.M. Gruner, Intrinsic curvature hypothesis for biomembrane lipid composition: a role for nonbilayer lipids, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 3665–3669.
[9] L.V. Chernomordik, J. Zimmerberg, Bending membranes to the task—structural intermediates in bilayer fusion, Curr. Opin. Struck. Biol. 5 (1995) 541–547.
[10] L. Yang, H.W. Huang, Observation of a membrane fusion intermediate structure, Science 297 (2002) 1877–1879.
[11] H. Ellens, D.P. Siegel, D. Alford, P.L. Yeagle, L. Boni, L.J. Lis, P.J. Quinn, J. Bentz, Membrane fusion and inverted phases. The modified stalk mechanism of lamellar/inverted phase transitions and its implications for membrane fusion, Biochemistry 28 (1989) 3692–3703.
[12] R.M. Epand, Diacylglycerols, lysolecithin, or hydrocarbons markedly alter the bilayer to hexagonal phase transition temperature of phosphatidylethanolamines, Biochemistry 24 (1985) 7092–7095.
[13] D.P. Siegel, J. Banschbach, D. Alford, H. Ellens, L.J. Lis, P.J. Quinn, P.L. Yeagle, J. Bentz, Physiological levels of diacylglycerols in phospholipid membranes induce membrane fusion and stabilize inverted phases, Biochemistry 28 (1989) 3703–3709.
[14] G. Basáñez, J.L. Nieva, E. Rivas, A. Alonso, F.M. Goñi, Diacylglycerol and the promotion of lamellar–hexagonal and lamellar–isotropic phase transition in lipids: implications for membrane fusion, Biophys. J. 70 (1996) 2299–2306.
[15] R.M. Epand, Virus replication inhibitory peptide inhibits the conversion of phospholipid bilayers to the hexagonal phase, Biosci. Rep. 6 (1986) 647–653.
[16] M.J.M. Darkes, S.M.A. Davies, J.P. Bradshaw, X-ray diffraction study on feline leukaemia virus fusion peptide and lipid polymorphism, FEBS Lett. 461 (1999) 178–182.
[17] M.J.M. Darkes, T.A. Harroun, S.M.A. Davies, J.P. Bradshaw, The effect of fusion inhibitors on the phase behaviour of *N*-methylated dioleoylphosphatidylethanolamine, Biochim. Biophys. Acta 1561 (2002) 119–128.
[18] A. Colotto, I. Martin, J.-M. Ruysschaert, A. Sen, S.W. Hui, R.M. Epand, Structural study of the interaction between the SIV fusion peptide and model membranes, Biochemistry 35 (1996) 980–989.
[19] C. Boulin, R. Kempf, M.H.J. Koch, S.M. McLaughlin, Data appraisal, evaluation and display for synchrotron radiation experiments—hardware and software, Nucl. Instrum. Methods Phys. Res. A249 (1986) 399–407.
[20] R.D.B. Fraser, T.P. MacRae, Unit cell and molecular connectivity in tendon collagen, Int. J. Biol. Macromol. 3 (1981) 193–200.
[21] R. Brasseur, M. Vandenbranden, B. Cornet, A. Burny, J.-M. Ruysschaert, Orientation into the lipid bilayer of an asymmetric amphipathic helical peptide located at the N-terminus of viral fusion proteins, Biochim. Biophys. Acta 1029 (1990) 267–273.
[22] J.P. Bradshaw, M.J.M. Darkes, T.A. Harroun, J. Katsaras, R.M. Epand, Oblique membrane-insertion of viral fusion peptide probed by neutron diffraction, Biochemistry 39 (2000) 6581–6585.
[23] S. Kamath, T.C. Wong, Membrane structure of the human immunodeficiency virus gp41 fusion domain by molecular dynamics simulation, Biophys. J. 83 (2002) 135–143.
[24] S.M.A. Davies, J.P. Bradshaw, R.M. Epand, Modulation of lipid polymorphism by the feline leukaemia virus fusion peptide: implications for the fusion mechanism, Biochemistry 37 (1998) 5720–5729.
[25] J.M. Seddon, Structure of the inverted hexagonal (H_2) phase and nonlamellar phase transitions of lipids, Biochim. Biophys. Acta 1031 (1990) 1–69.