Structural plasticity of the feline leukaemia virus fusion peptide: a circular dichroism study

Sarah M.A. Davies[a,*], Sharon M. Kelly[b], Nicholas C. Price[b], Jeremy P. Bradshaw[a]

[a] *Department of Preclinical Veterinary Sciences, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Summerhall, Edinburgh EH9 1QH, UK*

[b] *Department of Biological and Molecular Sciences, University of Stirling, Stirling FK9 4LA, UK*

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Abstract The secondary structure of the feline leukaemia virus (FeLV) fusion peptide was investigated using circular dichroism (CD). Our results show that this peptide can readily flip between random, α-helical and β-sheet conformations, depending upon its environment. The CD spectrum changes from one characteristic of random coil to predominantly β-sheet type, and finally to that showing the characteristics of α-helical structure on moving from an aqueous solvent, through several increasingly hydrophobic systems, to a highly hydrophobic solvent. Electron microscopy confirmed the presence of β structure. We propose that the structural plasticity demonstrated here is crucial to the ability of the fusion peptide to perturb lipid bilayers, and thus promote membrane fusion.

Key words: Spectrophotometry; Circular dichroism; Electron microscopy; Feline leukemia virus; Fusion peptide; Structural plasticity

1. Introduction

Membrane fusion, the merging of two distinct lipid bilayers to form one common bilayer, is essential to life, occurring many times daily within every animal cell [1]. Intercellular fusion is also important, for example between sperm and egg in the mammalian fertilisation process [2]. In addition, many intracellular parasites, including all enveloped animal viruses, utilise fusion to gain entry to their target cells, and thus cause disease [3]. However, despite the clear importance of fusion, the precise mechanisms involved in this process are still not known.

Enveloped viruses have specific envelope glycoprotein 'spikes', viral fusion proteins, which mediate fusion between the virus and its target cell [4]. Each fusion protein contains a fusion peptide, a span of relatively hydrophobic amino acids, which is thought to insert into the host cell target membrane and disrupt bilayer stability, so initiating the fusion process [5]. The active participation of fusion peptides in the fusion process, for example those of influenza virus and human immunodeficiency virus (HIV), has been shown by a variety of techniques including hydrophobic affinity labelling experiments [6] and site-directed mutagenesis studies [7,8]. Studies using synthetic peptides, which correspond to the sequences of viral fusion peptides, have helped to determine some of the molecular mechanisms involved in viral-mediated fusion. In particular, these synthetic peptides appear to be most useful for studies on the minimum and precise molecular and structural requirements for membrane destabilisation [9–11].

Translocation from an aqueous environment, such as extracellular fluid, to a lipid environment is likely to involve substantial structural alterations of a peptide. Many proteins and peptides insert into membranes, and concomitantly alter their own secondary structure, but they do not trigger huge changes in the arrangements of the lipid molecules in their target membranes.

There is much conflicting evidence on the active secondary structure of fusion peptides. A modelling study of several viral fusion peptides assumed that they completely formed α helices on membrane insertion [5]. However, Gallaher et al. have cautioned against the earlier assumption that all fusion peptides are 'sided' helices in their active form [12]. Indeed, the measles virus fusion peptide was found to adopt a conformation of 73% β sheet in one study [13], and, under different experimental conditions, fusion peptides from different HIV strains have been shown to be mainly α-helical [14] or conversely mainly β-sheet [15] in their active forms. Hepatitis B virus fusion peptide favours a β-sheet conformation in the presence of lipid vesicles [16], as does PH-30, a sperm fusion peptide involved in sperm-egg fusion [17]. However, none of these studies examines the same fusion peptide in a whole range of different solvent systems.

We wished to examine the secondary structure of the fusion peptide from the retrovirus feline leukemia virus (FeLV) in a variety of environments. The FeLV peptide contains significant numbers of amino acids that characteristically favour three different secondary structures, namely the α helix, the β sheet and a coiled conformation. The conclusion drawn from the various studies mentioned above is that fusion peptides change from one defined, inactive secondary structure to another different and equally well-defined active secondary structure. We speculated that this might lead to a large amount of dynamic structural flexibility for the FeLV fusion peptide. Indeed, computer modelling work has hinted that this might be an important factor for fusion peptides in general [18]. Since membrane fusion is a dynamic process, involving large changes in the component molecules, structural plasticity may be an essential property of these initiators of fusion.

2. Materials and methods

A 28-residue peptide (sequence EPISLTVALMLGGLTVGGIAAGVGTGTK), which corresponds to the common sequence of the amino-terminal fusion peptide of all documented strains of FeLV [19], was synthesised. A lysine (which is the next naturally occurring carboxy-terminal residue in the fusion protein) was included on the carboxy-terminus to increase peptide solubility. Peptide synthesis and purification were performed by Albachem Ltd, 26 Craigleith View,

*Corresponding author. Fax: (44) (131) 650-6576.
E-mail: sdavies@lab0.vet.ed.ac.uk

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Edinburgh EH4 3JZ, UK. The peptide was synthesised on an Applied Biosystems 430A instrument, using Fmoc chemistry with the side chain protecting groups selected as tBu (Ser, Thr) and OtBu (Glu). The completed peptide was cleaved with a solution of TFA/H_2O (95:5) plus scavengers (ethanedithiol/thioanisole/triisopropanesilicane) and the solution was evaporated under vacuum. The crude peptide was dissolved in 50% TFA/H_2O and purified by reverse phase HPLC, using a RPC4 (10×100 mm) column, eluting with a linear gradient from 10% acetonitrile in water (0.1% TFA) to 60% acetonitrile in water (0.1% TFA) over 30 min. The peptide was characterised by mass spectra (MALDI, PerSeptive Biosystem laserTec), amino acid analysis (LKB 4150 alpha amino acid analyser) and analytical HPLC using PRC4 (4.6×100 mm), RPC8 (4.6×220 mm) and Vydac C8 (4.6×250 mm) columns, running a linear gradient of 10% acetonitrile in water (0.1% TFA) to 90% acetonitrile in water (0.1% TFA) over 30 min. The assembly of the peptide was reasonably efficient but the purification process was found to be very difficult. The peptide was found to be very insoluble; accordingly 50% TFA was added to take it into solution. However, a very broad peak without any resolution was obtained under normal eluting conditions, unless a very dilute solution was applied. Thus the purification could only be carried out batchwise on a small scale (0.5–1.0 mg per run).

The fusion peptide was added at a concentration of 1 mg/ml to (a) 2 M guanidine/50% ethanol, a solvent often used for peptide addition to fusion assays, (b) trifluoroethanol (TFE), and (c) hexafluoroisopropanol (HFIP). These samples were then diluted 1:1 with distilled water, giving a final peptide concentration of 0.5 mg/ml. Peptide was also added to sodium dodecylsulphate (SDS) at concentrations (c) 3 mM, (d) 6 mM in distilled water, to give a final peptide concentration of 0.5 mg/ml. The resulting suspensions were sonicated in a bath sonicator until the peptide dissolved. All chemicals were supplied by Sigma Chemical Co., UK.

CD spectra were recorded using a JASCO J-600 spectropolarimeter, over the wavelength range 195–260 nm, in a cell of path length of 0.02 cm, at 25°C. At least four spectra were averaged for each sample. Analysis of the spectra for secondary structure content utilised the CONTIN procedure of Provencher and Glöckner [20] to determine the amounts of α helix and β sheet present, and the method of Chang et al. [21] was used to verify the overall trend in α helix contents.

Negative staining transmission electron microscopy was used to examine the peptide samples for the presence of β structure. The peptide solutions were dried onto plastic-coated carbon grids, and then negatively stained using 4% sodium phosphotungstate at pH 7.2. A Philips 400 TEM was used, at 80 kV, and ×60 000 magnification. Negative controls were performed by staining an empty plastic-coated carbon grid, and also by staining the corresponding pure solvent.

3. Results

The far ultraviolet CD spectra of the FeLV fusion peptide in the various solvents are shown in Fig. 1. Satisfactory data could be obtained down to 195 nm; below this wavelength the noise levels prevented accurate determination of ellipticity. The CD spectra exhibit an increase in α-helical character on moving from 1 M guanidine/25% ethanol, through a selection of increasingly hydrophobic solvents, to 50% HFIP. In the guanidine/ethanol sample, the peptide shows a spectrum characteristic of random coil secondary structure, which is to be expected for a hydrophobic peptide in a polar solvent containing a chaotropic agent. The critical micellar concentration (CMC) of SDS is 8 mM at 20°C. In 3 mM SDS, the CD spectrum is typical of a β sheet, but as the concentration of SDS increases towards the CMC, the spectrum indicates that some α-helical structure is present. The CD spectrum in 50% TFE shows considerably more α-helical character and this is even more pronounced in 50% HFIP. Analysis of the spectra gives the percentages of α helix and β sheet in each sample listed in Table 1. The exact values of these estimates should be viewed with caution, since (a) ellipticity data could only be collected down to 195 nm instead of 190 nm, which is the preferred lower limit, and (b) it may be inappropriate to apply the methods of analysis which have been derived for proteins to oligopeptides. Moreover, the amounts of helix present may well be underestimated, as up to twofold reductions in ellipticity have been found in membrane-bound proteins [22]. However, an overall trend from random coil through predominantly β sheet to significant amounts of α helix can clearly be seen.

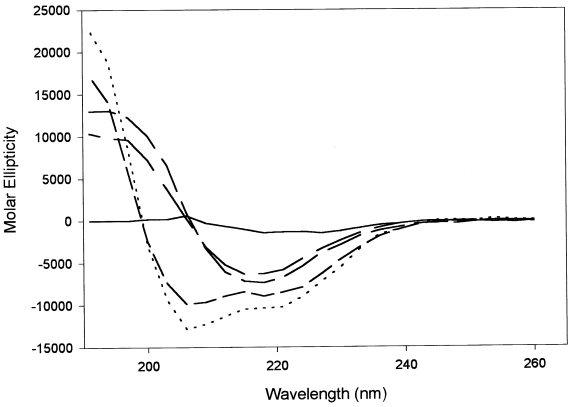

Fig. 1. CD spectra of the FeLV fusion peptide in a variety of solvents. Solid line: in 1 M guanidine/25% ethanol; short dash: in 50% TFE; dotted line: in 50% HFIP; long dash: in 3 mM SDS; medium dash: in 6 mM SDS.

Fig. 2 shows electron micrographs of FeLV peptide in 3 mM SDS, FeLV peptide in 50% HFIP and negative control: stained empty grid. Beta fibrils are visible in the 3 mM SDS sample, and were only seen when there was little evidence of α-helical content by CD spectroscopy (other data not shown).

Table 1
Percentages of FeLV peptide secondary structure, as calculated by the methods of Provencher and Glöckner [20] and Chang et al. [21]

Sample FeLV peptide (0.5 mg/ml) in:	% Secondary structure as calculated by the methods of:	
	Provencher and Glöckner [20] (helix, sheet)	Chang et al. [21] (helix)
1 M guanidine in 25% ethanol	0, nd[a]	0
50% TFE	20, 36	16
50% HFIP	30, 35	22
3 mM SDS	2, 57	11
6 mM SDS	12,47	14

[a]The value for the sheet content of this sample could not be determined using the method of Provencher and Glöckner [20] without an unacceptably large error.

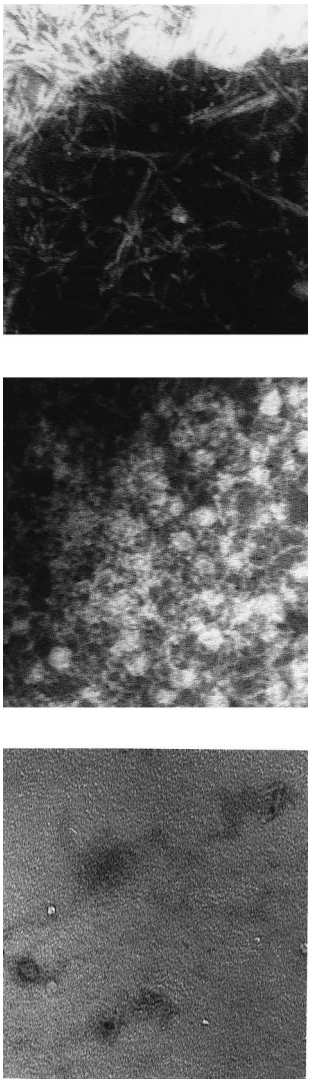

Fig. 2. Electron micrographs of (top) FeLV peptide in 3 mM SDS, (middle) FeLV peptide in 50% HFIP and (bottom) stained grid control.

These β fibrils appeared long and unbranched and were arranged in a meshwork.

4. Discussion

A limited amount of Fourier transform infrared spectroscopy has been performed on the fusion peptides of two retroviruses simian immunodeficiency virus (SIV) [23] and HIV [24]. These peptides were found to show a partial conversion from a β-sheet conformation in an aqueous solvent to an α helix in a lipid environment. Filtration to remove the non-lipid bound peptide yielded an increase in the amount of recorded α helix. Certainly the increase in the proportion of α helix which we observed for the FeLV peptide on increasing the solvent hydrophobicity agrees with these data. However, the assumption from the SIV and HIV studies was that the α-helical form is the single, fusion-active conformation. Work on other fusion peptides [15,16] and a different study on the HIV peptide [14] have all suggested that the β conformation may be the fusion-active state.

Fusion is an extremely rapid, multi-step process, and so structural measurements are obtained mainly on fusion end products. Thus only the final peptide conformations are observed, but none of the intermediate changes in conformation, which are adopted during the different stages of fusion, are seen. We propose that structural flexibility, rather than the rigid adoption of a particular secondary structure, may be a key property of fusion peptides. Membrane fusion is a dynamic process involving large structural changes in the participating molecules. We therefore suggest that it is possible that the critical feature of these catalytic peptides is their ability to 'flip' between different secondary structures extremely rapidly, rather than the adoption of any single, well-defined secondary structure. The energy barriers between these different secondary conformations must be low: the peptide must be able to adopt, at least transiently, these various structures, which presumably represent local energy minima. Clearly the secondary structures adopted under certain conditions may vary between individual fusion peptides, due to differences in their primary structures. However, previous computer modelling has hinted that structural flexibility may be a common property of fusion peptides [18]. Here we provide direct experimental proof of such flexibility for one fusion peptide. It is interesting to note that there is an analogy with the structural plasticity previously observed in a pathogenic prion peptide, PrP(106–126) [25], which has been implicated in prion-related diseases.

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