



Neutron diffraction studies of viral fusion peptides

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Abstract

Membrane fusion plays a vital role in a large and diverse number of essential biological processes. Despite this fact, the precise molecular events that occur during fusion are still not known. We are currently engaged on a study of membrane fusion as mediated by viral fusion peptides. These peptides are the N-terminal regions of certain viral envelope proteins that mediate the process of fusion between the viral envelope and the membranes of the host cell during the infection process. As part of this study, we have carried out neutron diffraction measurements at the ILL, BeNSC and Chalk River, on a range of viral fusion peptides. The peptides, from simian immunodeficiency virus (SIV), influenza A and feline leukaemia virus (FeLV), were incorporated into stacked phospholipid bilayers. Some of the peptides had been specifically deuterated at key amino acids. Lamellar diffraction data were collected and analysed to yield information on the peptide conformation, location and orientation relative to the bilayer. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Enveloped viruses, such as HIV and influenza, infect host cells by fusing their phospholipid envelope with one of the membranes of the host cell. This membrane may be either the plasma membrane or an endosomal membrane after endocytosis of the virion. The fusion event is mediated by membrane glycoproteins in the viral envelope that bring about the merging of virion and host cell membranes. Synthetic peptides corresponding to the N-terminal region of several viral glycoproteins have been demonstrated to be fusion active and capable of inducing fusion between lipid vesicles. These so-called ‘fusion peptides’ have been the focus of attention of a number of

studies that have attempted to characterise the sequence of molecular events that bring about membrane fusion.

Membrane fusion seems to follow a multistep pathway, through several uncertain, highly bent lipid intermediate conformations, resulting in fusion pore formation [1]. The overall kinetics of fusion have been characterised extensively [2,3]; less is known about the subtle structural changes involved. Several models have been devised for putative lipid intermediates involved in fusion [4,5]. It has been suggested that fusion peptides catalyse fusion by increasing the negative curvature of the target membrane’s outer monolayer [6–8].

We have been using a number of biophysical techniques to probe the fusion mechanism of three viral fusion peptides. These peptides correspond to the N-terminal regions of envelope glycoproteins from simian immunodeficiency virus (SIV), influenza A and feline leukaemia virus (FeLV). Neutron diffraction has played a key role in these studies. Here we present lamellar neutron diffraction data from specifically deuterated SIV

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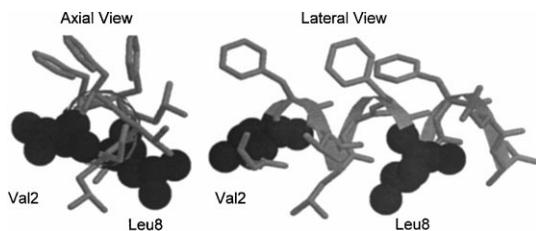


Fig. 1. Model of the SIV peptide, assuming α -helical structure. The two amino acids labelled with deuterium for this neutron diffraction study are indicated.

peptide, in a highly aligned multi-bilayer stack of phospholipids.

2. Materials and methods

2.1. Sample preparation

1,2-dioleoyl-*sn*-glycero-phosphocholine (DOPC) was purchased from Avanti Polar Lipids (Birmingham, AL) and used without further purification. SIV fusion peptide was synthesised and purified by Albachem (Edinburgh, UK) to the sequence in the Swiss Protein Data Bank: Gly-Val-Phe-Val-Leu-Gly-Phe-Leu-Gly-Phe-Leu-Ala. Three batches of the peptide were produced, one undeuterated, one deuterated at Valine 2 (8 deuterons) and one deuterated at Leucine 8 (10 deuterons). Fig. 1 shows the peptide and sites of deuteration.

Samples containing 20 mg of DOPC were deposited on silicon wafers, approximately 50 mm \times 25 mm, using an artist's airbrush. The spraying solvent was chloroform. Some samples contained SIV fusion peptide at a level of 1.0 mol %. The wafers were placed in a vacuum desiccator for 12 h to remove all traces of the solvent, before being hydrated in a desiccator above water of 0%, 33%, 67% or 100% $^2\text{H}_2\text{O}$.

2.2. Neutron data collection

Neutron diffraction measurements were carried out on the N5 diffractometer on the NRU reactor at Chalk River Laboratories, Canada. The sample environment was an aluminium can. A temperature of 25°C was achieved by circulating water through an integral water jacket, and a relative humidity of 92% was obtained by placing a saturated solution of potassium nitrate (KNO_3) at the base of the can.

$^2\text{H}_2\text{O}/\text{H}_2\text{O}$ exchange was used to provide additional phase information. Each sample was run at four $^2\text{H}_2\text{O}$ concentrations, 0%, 33%, 67%, and 100% $^2\text{H}_2\text{O}$. In

order to control the isotopic composition of the hydrating water, each sample was rehydrated at 25°C in an atmosphere of 100% relative humidity for at least 24 h before being transferred to the sample can. At each subsequent change of solvent, the sample was first dried out, then rehydrated in an atmosphere saturated with water at the new isotopic composition for at least 24 h. The scanning protocol consisted of sequential θ (sample angle) scans around the predicted Bragg angle for each order.

2.3. Data analysis

The data took the form of a two-dimensional rocking curve for each order of diffraction. The background around each peak was fitted and subtracted using Peakfit (Jandel Scientific Software GmbH), a commercial spreadsheet and graphing package. Gaussian curves were then fitted to the Bragg reflections and the angular position, width and area of each peak recorded. Absorption and Lorentz corrections were applied and the intensities square-rooted to produce structure factor amplitudes. The relative scaling of the different data sets and the phases of each of their orders were determined by least-squares fitting to straight line functions. The whole procedure has been described previously [9].

The d -repeat (lamellar repeat distance) and instrument offset were calculated by least-squares fitting of Q to the order number for all lamellar peaks observed for each sample.

The data were placed on a 'relative absolute' [10–12] scale using the known neutron scattering lengths of ^2H and H to calculate the scattering differences between $^2\text{H}_2\text{O}$ and H_2O samples, and between deuterated and undeuterated peptides. This method requires knowledge of the molar percentage of water in the samples, which was determined as previously described [13].

3. Results

Neutron scattering density profiles, calculated by Fourier transformation of the corrected and scaled structure factors are shown in Fig. 2. The difference plots show the distribution of deuterium label across the bilayer normal. This takes the form of two discrete populations for the $^2\text{H}_{10}$ – Leu8 label and one wide population for the $^2\text{H}_8$ – Val2 label (each label site is also reflected in the centrosymmetric unit cell).

The d -repeat was 51.05 Å. Gaussian distributions were fitted to the observed differences between deuterated and undeuterated SIV fusion peptide, the variables being height, width and position along the bilayer normal. The fitting process was carried out in reciprocal (diffraction)

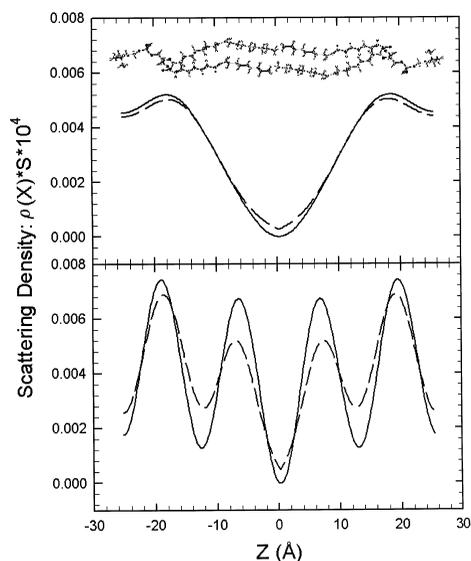


Fig. 2. (a) Neutron scattering length density profiles of deuterium label in 1% (mol) ($^2\text{H}_8 - \text{Val}2$)-SIV fusion peptide (solid line), calculated by subtracting structure factors for undeuterated SIV fusion peptide in DOPC bilayers from corresponding structure factors for ($^2\text{H}_8 - \text{Val}2$)-SIV fusion peptide. Also shown is a pair of Gaussian distributions fitted, in reciprocal space, to the difference structure factors (dotted line). (b) Neutron scattering length density profiles of deuterium label in 1% (mol) ($^2\text{H}_{10} - \text{Leu}8$)-SIV fusion peptide (solid line) and fitted Gaussian peaks (dotted line). The structure factors for bilayers hydrated with 8% $^2\text{H}_2\text{O}$ were used, since water of this isotopic has a net neutron scattering length density of zero. The data have been scaled using the relative absolute method [10–12]. Phospholipid molecules are shown above the profiles to orientate the scattering densities.

space by comparing the calculated structure factors of each model to the observed difference structure factors. The results of the model-fitting are summarised in Table 1.

Table 1

Gaussian models of deuterium label distribution of 1% (mol)($^2\text{H}_8$ -Val2)-SIV fusion peptide and ($^2\text{H}_{10}$ -Leu8)-SIV fusion peptide in bilayers of DOPC. The position, width and size of Gaussian distributions were fitted, in reciprocal space, to difference neutron structure factor. Five orders of diffraction were used in the fitting procedure

Site	Parameter	Valine 2 (\AA°)	Distribution	Leucine 8 (\AA°)	Distribution (%)
1	Position ^a	16.8 ± 0.1	100%	7.1 ± 0.1	40.3 ± 1.4
	Width ^b	19.8 ± 0.1		9.2 ± 0.5	
2	Position ^a	—	—	18.9 ± 0.1	59.7 ± 1.4
	Width ^b	—		10.3 ± 0.3	

^aThe position of each label site is expressed as distance from the centre of the bilayer.

^bThe width is the full-width at $1/e$ height.

4. Discussion

Fourier transform infrared (FTIR) spectroscopy has shown that the SIV fusion peptide is predominantly α -helical, at these concentrations, in DOPC [14]. The $^2\text{H}_8 - \text{Val}2$ difference profiles (Fig. 2a) show a single, wide distribution of label, close to the water–bilayer interface. This supports the suggestion that at least the N-terminal of the peptide is located close to the bilayer surface. The fusion peptide could be lying along the bilayer surface, with its axis approximately parallel to the bilayer. However, the wide Gaussian peaks in Fig. 2a show that the peptide is either relatively disordered or has more than one location relative to the bilayer.

The $^2\text{H}_{10} - \text{Leu}8$ data (Fig. 2b) clearly show two discrete label sites on each side of the bilayer. This may be taken to indicate that the SIV peptide adopts two orientations or locations, one positioning the C-terminus close to the bilayer surface, and one involving insertion of this region into the fatty-acyl region. In both of these orientations the N-terminus would be close to the bilayer surface, thereby explaining the single, wide, label peak for the $^2\text{H}_8 - \text{Val}2$.

Modelling work on several viral fusion peptides by Brasseur [6] has suggested they insert into phospholipids at an oblique angle of $55\text{--}60^\circ$. Moreover, there is indirect evidence connecting the membrane activity of SIV fusion peptide analogues and their mode of insertion into the phospholipid bilayer. Fusogenic activity is only observed when the peptide inserts into the bilayer with an oblique orientation [15]. Do these neutron data support an oblique insertion? At this stage, detailed model-fitting is not yet completed, but simple geometry allows us to compare our data to the proposed insertion angle for the SIV peptide of 55° , relative to the bilayer surface [6]. Assuming α -helical conformation of the peptide, the two label sites would be separated by approximately 6.0\AA . From Table 1 we see that the deepest label site for $^2\text{H}_{10} - \text{Leu}8$ is centred at 7.1\AA from the bilayer centre. If the peptide inserts at 55° then the $^2\text{H}_8 - \text{Val}2$ label should

be centred 4.9 Å further out, at 12.0 Å from the centre of the bilayer. This is entirely consistent with our results and suggests that the wide ²H8 – Val2 Gaussian may actually be two overlapping populations of label, one corresponding to the obliquely inserted peptide, and one to peptide lying parallel to the bilayer surface. It is hoped that more detailed model-fitting will help to clarify this.

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