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A synthetic peptide corresponding to a conserved heptad repeat domain is a potent inhibitor of Sendai virus–cell fusion: an emerging similarity with functional domains of other viruses

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A series of peptides derived from three domains within the fusion protein of Sendai virus was synthesized and examined for their potential to inhibit the fusion of the virus with human red blood cells. These domains include the 'fusion peptide' and two heptad repeats, one adjacent to the fusion peptide (SV-163) and the other to the transmembrane domain (SV-473). Of all the peptides tested, only SV-473 was highly inhibitive. Using fluorescently-labelled peptides, the mechanism through which the SV-473 peptide inhibits the haemolytic activity of the virus was investigated. The results suggest that interactions of the active peptide with virion elements and lipid membranes are involved. Since it has recently been found that synthetic peptides corresponding to putative coiled-coil domains of the human immunodeficiency virus (HIV) type 1 transmembrane protein gp41 are potent inhibitors of HIV, we discuss the general property of virus-derived coiled-coil peptides as inhibitors of viral infection.

Keywords: coiled-coils/inhibition/Sendai virus/synthetic peptides

Introduction

Fusion events are associated with the entry of enveloped viruses into host cells (Blumenthal, 1987; Stegmann *et al.*, 1989a,b; Hoekstra, 1990). Activation of viral fusion proteins by proteolytic cleavage results in the formation of new N-terminal regions. A comparison of such newly formed N-termini in various virus families indicated that these regions are exceptionally hydrophobic and highly conserved within families (Blumberg *et al.*, 1985; Wiley and Skehel, 1987). Site-specific mutations in these regions of various viruses severely modify their fusion activity, indicating the importance of these domains in mediating the fusion process (Gething *et al.*, 1986; Stegmann *et al.*, 1989a,b; Bosch *et al.*, 1989; Freed *et al.*, 1990; Horvath and Lamb, 1992). It has been suggested that the fusion peptide interacts with the target membrane (Novick and Hoekstra, 1988), possibly penetrating it and thus inducing the fusion.

Recent reports indicated that other domains within viral fusion proteins are also important in mediating the fusion event. For example, the cytoplasmic region was found to be necessary for complete fusion in influenza virus (Kemble *et al.*, 1994). Furthermore, various heptad repeat domains that have the potential to form a coiled-coil structure were identified and found to be necessary for fusion-related conformational changes in influenza virus (Carr and Kim, 1993; Bullough *et al.*, 1994), and they may have a similar role in HIV as well (Wild *et al.*, 1992). Moreover, a synthetic peptide corresponding to a leucine zipper-like structure in influenza virus was found to interact with lipid membranes, indicating a potential role of this domain in membrane apposition (Yu *et al.*, 1994).

Synthetic peptides corresponding to various domains within the human immunodeficiency virus type 1 (HIV-1) envelope protein gp41 were recently found to display various extents of anti-viral activity (Wild *et al.*, 1992, 1994a; Jiang *et al.*, 1993a,b; Nehete *et al.*, 1993; Slepushkin *et al.*, 1993). The anti-viral activity of some of these peptides was suggested to be due to their ability to accurately model and interact with functional domains of the protein (Wild *et al.*, 1994a).

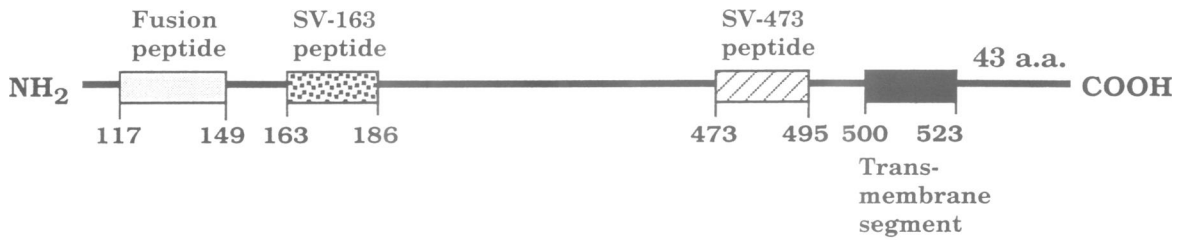
Sendai virus belongs to the family of paramyxoviruses, contains two spike glycoproteins (HN and F) and fuses at neutral pH with the cellular plasma membrane (Lamb, 1993). The F protein is oligomeric, probably a trimer (Russell *et al.*, 1994), and contains two heptad repeats (see Figure 1). One is adjacent to the fusion peptide and the second is adjacent to the transmembrane anchoring domain (Chambers *et al.*, 1992). Mutations in both domains were found to interfere with the fusogenic potential of the F protein (Buckland *et al.*, 1992; Sergel-Germano *et al.*, 1994). To investigate whether the inhibitory potential of peptides modelled after functional domains of fusion proteins is a general property of viral systems, and to obtain further information on virus penetration mechanisms, we synthesized a series of peptides derived from the fusion protein F of Sendai virus and investigated their inhibitory potential. Three peptides derived from the fusion peptide (the wild-type, its G12A variant and a randomized peptide) and their charge-reversed analogues were investigated. In addition, two peptides corresponding to heptad repeat domains were studied (Figure 1).

Among all the peptides tested, only a peptide modelled after a heptad repeat domain adjacent to the transmembrane region (SV-473) was highly inhibitive. Using fluorescently-labelled peptides we obtained information on its membrane and viral association properties. The results of the present study are compared with those of related studies on the inhibitory potential of HIV-1 peptides, and possible mechanisms of inhibition are discussed.

Results

Peptides investigated for inhibitory potential

A peptide representing the N-terminal 33 amino acids of the fusion protein F_1 of Sendai virus, its Gly12 to Ala12



WT	FFGAVIGTIALGVATSAQITAGIALAEAREAKR
G12A	FFGAVIGTIALAVATSAQITAGIALAEAREAKR
Ran	VILEQRAFAVGGAILTSKFAIGGRTAAIATAEA
SV-163	KSVELLQNAVGEQILALKTLQDFV
SV-473	FLQDSKAELEKARKILSEVGRWY

Fig. 1. Schematic representation of the F_1 fusion protein of Sendai virus and the designations and sequences of the peptides used.

analogue, which corresponds to the fusion peptide of a variant with enhanced fusogenic activity (Horvath and Lamb, 1992), a scrambled fusion peptide (with the same amino acid composition as the native peptide but with randomized sequence) and their charge-modified analogues were synthesized. In addition, two peptides corresponding to heptad repeat domains were synthesized and fluorescently-labelled to investigate their interaction with virions and lipid membranes. The sequences of the peptides, their designations and a schematic representation of the fusion protein F_1 of Sendai virus are given in Figure 1. All these peptides were investigated for their potential to inhibit Sendai virus–cell fusion. The virus-induced haemolysis of human red blood cells was used as a measure of the activity of Sendai virus fusion, as it is generally accepted that haemolysis results from the fusion of the virus with the target membrane (Loyter and Volsky, 1982).

Synthetic analogues of the fusion peptide lack inhibitory potential

It was reported that the fusion peptide of HIV-1 can inhibit viral infection (Slepushkin *et al.*, 1993). Therefore, we investigated the inhibitory potential of the fusion peptide of Sendai virus, its fusion-enhanced analogue (G12A) (Rapaport and Shai, 1994) and a randomized peptide. Furthermore, as oligomerization of polypeptides was hypothesized to occur during the fusion process (Freed *et al.*, 1992; Lamb, 1993), we speculated that an analogue with complementary charge could be an inactive peptide that would co-aggregate with the active peptide and inhibit the ability of the latter to induce fusion. To examine this possibility, analogues in which the N-terminal amino acids were succinylated in order to reverse their charge were also investigated.

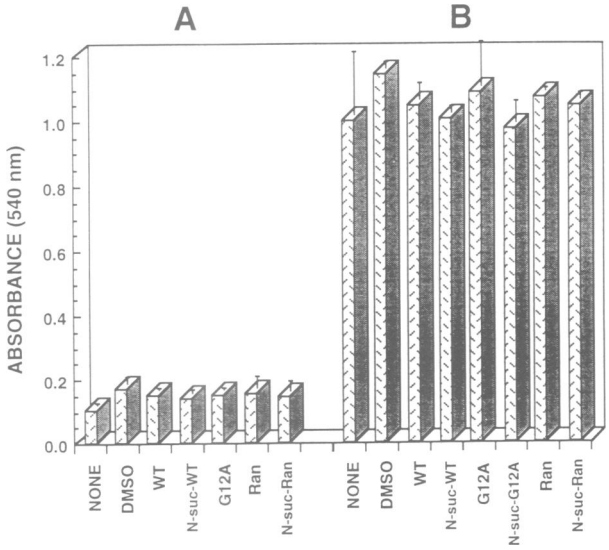


Fig. 2. The haemolytic potential of the fusion peptide of Sendai virus and its analogues, and the effect on Sendai virus-induced haemolysis. As the peptides were dissolved in dimethylsulfoxide (DMSO), the effect of DMSO alone is also shown. (**A**) Peptides (4 μM) were added to duplicate samples of 1 ml of 4% red blood cells (RBC) at 4°C. The mixture was incubated for 30 min at 4°C followed by 1 h at 37°C. At the end of the incubation period the samples were centrifugated at 5700 *g* for 10 min and the absorbance of two samples from the supernatant was measured at 540 nm. (**B**) Virions (100 HAU) were added to duplicate samples of 1 ml of 4% RBC at 4°C. The solution was incubated on ice for 30 min, washed twice with PBS to remove unattached virions, and then peptides were added. The rest of the experiment was as described in (A).

None of the peptides was haemolytic up to a concentration of 6 μM and none was able to inhibit the haemolytic activity of the virus in the conditions tested (Figure 2). In

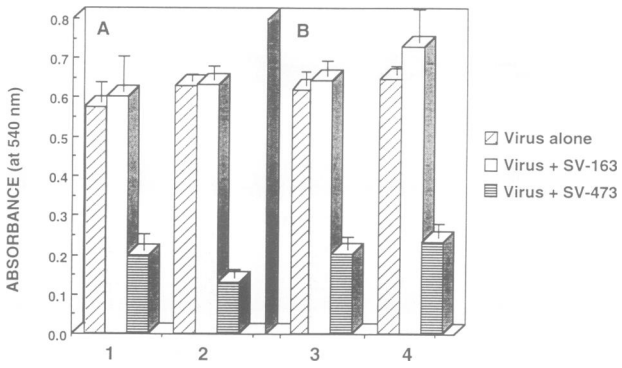


Fig. 3. The inhibition potential of the 'heptad repeat' peptides under various conditions. In all cases peptides (8.3 μM) were added to duplicate samples of Sendai virus (100 HAU). The final incubation was always at 37°C for 30 min, followed by centrifugation at 5700 *g* for 10 min. (**A**) Peptides were added to virions in 200 μl PBS and were incubated for 10 min at 4°C (1) or at RT (2). RBC (250 μl of 4%) were then added and further incubated for 15 min at 4°C (1) or at RT (2). (**B**) (3) Peptides were added to 450 μl RBC (2.2%) and were incubated for 10 min at 4°C. Virions were then added and incubated at 4°C for 15 min. (4) Virions were added to 500 μl of 2% RBC at 4°C. The solution was incubated on ice for 30 min, washed twice with PBS to remove unattached virions and then peptides were added and further incubated for 10 min at 4°C.

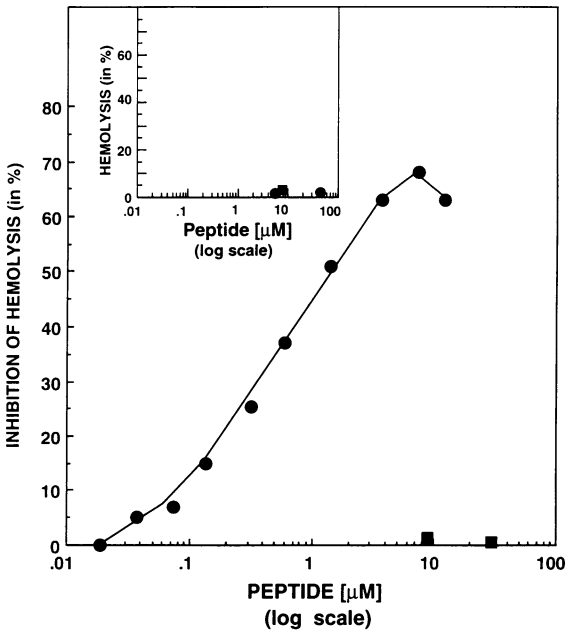


Fig. 4. Dose–response of the inhibition potential of the SV-473 and SV-163 peptides. Various amounts of a peptide were added to duplicate samples of virions in 250 μl PBS and incubated for 15 min at RT. RBC (250 μl of 8%) were then added and incubated at 37°C for 30 min. Inset: the haemolytic activity of the peptides alone as expressed in percent from the activity of the virus. SV-163, closed squares; SV-473, closed circles.

a previous study, the synthetic peptides corresponding to the native fusion peptide and its G12A analogue were found to interact with and fuse lipid vesicles (Rapaport and Shai, 1994). In contrast, the succinylated peptides did not induce the fusion of liposomes, nor did they inhibit peptide-induced liposome fusion by the native peptides (D.Rapaport and Y.Shai, in preparation).

SV-473 is a potent inhibitor of Sendai virus

Among the two heptad repeat peptides investigated, only SV-473, which is modelled after a domain adjacent to the transmembrane region, was found to be a potent inhibitor of viral activity (Figure 3). The inhibitory activity of SV-473 was similar under all the experimental conditions tested. The peptide was highly inhibitive, whether it was incubated with the virus at room temperature (RT) or at 37°C (Figure 3A). A similar extent of inhibition was observed if the peptide was added to the erythrocytes prior to the addition of the virions, or to a mixture of virions and RBC at 4°C (Figure 3B). The first step in the penetration process is the attachment of the virus to the RBC, which can take place even in the cold (4°C), a condition under which fusion is blocked (Hoekstra and Klappe, 1986). Therefore, the results presented in Figure 3B indicate that the peptide interferes with the fusion process after the attachment step.

The inability of SV-163 peptide to inhibit the virus-mediated haemolysis of erythrocytes could not be explained by the fact that it causes haemolysis by itself, as both peptides were found to be devoid of haemolytic activity in the concentration range tested (inset to Figure 4). The dose–response inhibitory activity of SV-473 was tested and the results are depicted in Figure 4. The results with SV-163 are also presented for comparison. The figure shows that at concentrations as low as 40 nM some inhibition takes place and that 50% inhibition occurs at ~1 μM peptide. The peptide exhibited its maximal inhibition (70%) at a concentration of 7.5 μM and higher concentrations of the peptide did not result in an increase of inhibition. Instead, a lower inhibitory effect was observed, which could indicate the occurrence of aggregation of the peptide in solution at this concentration (see next paragraph).

Mechanism of inhibition

The aggregational state of the peptides in solution. To gain insight into the mechanism of inhibition we labelled both SV-163 and SV-473 selectively at their N-terminal amino acids with a fluorescent probe, rhodamine or NBD. The biological activity of the labelled analogues was very similar to that of the unlabelled peptides (data not shown). The tendency of the peptides to self-associate in the solution was tested utilizing the sensitivity of the fluorescence of the rhodamine probe to self-quenching. The fluorescence intensity of the rhodamine-labelled peptides was measured as a function of their concentration in buffer at 37°C. The results revealed a deviation from linear increase in the fluorescence intensity above a concentration of 2 μM (data not shown), suggesting the beginning of oligomerization in solution.

The interaction of the peptides with phospholipid membranes. The potential of the peptides to interact with phospholipid membranes was investigated using the NBD-labelled peptides. The emission properties of the NBD probe are sensitive to the environment, exhibiting an increase in quantum yield and a blue shift in the emission maximum when the probe is relocated to a more hydrophobic milieu. These properties have been used before to investigate the interaction of various peptides with membranes (Frey and Tamm, 1990; Rapaport and Shai,

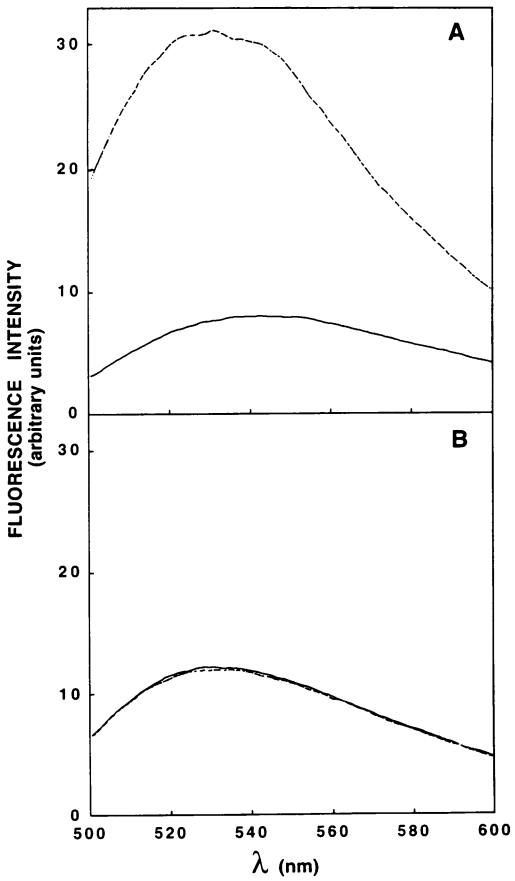


Fig. 5. Fluorescence emission spectra of NBD-labelled peptides in buffer or in the presence of liposomes. Spectra of 0.55 μM of NBD–SV-473 (**A**) and NBD–SV-163 (**B**) were determined in buffer composed of 150 mM NaCl, 10 mM sodium phosphate, pH 7.3 at 37°C, before (continuous line) and after (dashed line) the addition of 190 μM SUV composed of PS/PE/PC (2.8:1.5:1 molar ratio). The excitation wavelength was set at 465 nm, and emission was scanned from 500 to 600 nm.

1991). In our study, when negatively charged liposomes composed of egg phosphatidylcholine/egg phosphatidylethanolamine/phosphatidylserine (PC/PE/PS) were added to a solution containing NBD–SV-163 at 37°C no change in the emission spectra of the probe was observed (Figure 5B). However, the addition of the liposomes to a solution containing NBD–SV-473 caused a large increase in the fluorescence intensity of the NBD moiety concomitant with a blue shift of the emission maximum from 545 to 530 nm (Figure 5A). This blue shift suggests that the NBD probe is located at the surface of the membrane (Chattopadhyay and London, 1987). Similar results were observed with the zwitterionic PC lipids which better resemble the composition of the outer membrane of human erythrocytes (data not shown).

Enzymatic digestion of membrane-bound SV-473. Further support for the surface location of membrane-bound NBD–SV-473 comes from the finding that the peptide is susceptible to digestion by proteinase K when it is bound to lipid vesicles (Figure 6). The enzymatic cleavage of the peptide resulted in the formation of small peptidic fragments with much reduced membrane affinity. Therefore, the addition of the enzyme to membrane-bound peptide resulted in a dramatic reduction in the fluorescence intensity as the

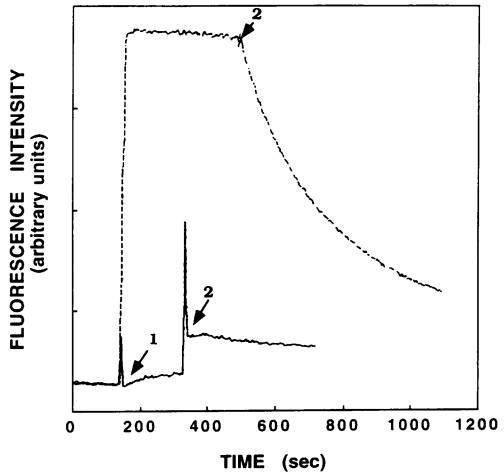


Fig. 6. Accessibility of NBD–SV-473 to proteolytic digestion. The fluorescence intensity of the labelled peptide was monitored at 530 nm (slit 8 nm) with the excitation at 465 nm (slit 10 nm). The experiment was done in 400 μl buffer composed of 150 mM NaCl, 10 mM sodium phosphate, pH 7.3 at 37°C, and lipid vesicles composed of PS/PE/PC (2.8:1.5:1 molar ratio) were used. In the first experiment 190 μM lipid vesicles were added at the first time point to labelled peptide (0.55 μM) followed by 4 μl of proteinase K solution (0.25 μg/μl) at the second time point (dashed line). In the other experiment proteinase K was added first and only later were the liposomes added (continuous line).

probe is relocated to the aqueous solution. As a control experiment, we first added the proteolytic enzyme to the peptide, and only then added the lipid vesicles. Under these conditions only a minor increase in fluorescence was observed (Figure 6). Other membrane-interacting peptides that were found to be buried within the lipid core were not cleaved by this enzyme (Pouny *et al.*, 1992; Gazit *et al.*, 1994).

The interaction of the peptides with Sendai virus. The ability of NBD–SV-473 to interact with Sendai virions was investigated. First, virions were added to a solution containing the labelled peptides, but no change in the fluorescence intensity of the NBD probe was observed (Figure 7). The fluorescence of NBD–SV-163 was also not affected by the virions (data not shown). These results suggest that in the presence of the virus the peptides are not relocated to a hydrophobic environment. However, this experiment cannot rule out the possibility that the peptides are attached to the spikes of the virus while the NBD-labelled N-terminus is exposed to the aqueous solution. To clarify that point, liposomes were added to the virus–peptide mixture and the resulting fluorescence intensity was compared with that when liposomes were added to NBD–SV-473 alone. The increase in the fluorescence of the NBD–SV-473 when the liposomes were added after the addition of the virus, was only half that obtained without the virions (Figure 7). Hence, we can conclude that at least a fraction of the SV-473 peptide was attached to the virions, and that this attachment prevented them from interacting with the liposomes. Interestingly, when the virions were added after the liposomes, a slow decrease in the fluorescence of the NBD was observed, thus giving further support for surface attachment to the liposomes and for reversibility of the binding process. Furthermore, incubation of the virions at

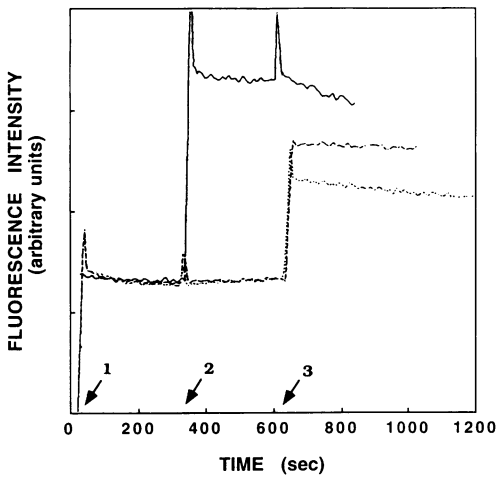


Fig. 7. The effect of Sendai virions on the interaction of NBD–SV-473 with membranes. The fluorescence intensity of the labelled peptide was monitored at 530 nm (slit 8 nm) with the excitation at 465 nm (slit 10 nm). The experiment was done in 400 μl buffer composed of 150 mM NaCl, 10 mM sodium phosphate, pH 7.3 at 37°C. In all cases labelled peptide (0.33 μM) was added to the solution at the first time point. In the first experiment 190 μM PC LUVs were added at the first time point followed by 5 μl of Sendai virions (50 HAU) at the second time point (continuous line). In the second case virions were added first and lipid vesicles were added at the second time point (dotted line), and in the last case virions that were heated to 65°C for 20 min were added first followed by lipid vesicles (dashed line).

65°C for 20 min (conditions that are known to reduce the activity of the virus; Nussbaum *et al.*, 1984), caused a reduction in the ability of the virus to bind NBD–SV-473, as revealed by the higher increase in the fluorescence of NBD after the addition of the liposomes (Figure 7).

Discussion

A search for a common mechanism of inhibition

Several synthetic peptides modelled after functional regions of the HIV-1 transmembrane protein gp41 have been found to possess anti-viral activity (Jiang *et al.*, 1993a,b; Slepushkin *et al.*, 1993; Wild *et al.*, 1992, 1994a). As the structural features of these domains are highly conserved (Gallaher, 1987), and since the mechanism by which these peptides inhibit HIV infection is not fully understood, we performed a study to assess whether such domains are also effective against the fusion process of Sendai virus. Such a comparative study was undertaken to gain information about the features of the fusion process of enveloped viruses in general.

Peptides corresponding to the fusion peptide lack inhibitory potential

A 22mer peptide corresponding to the N-terminus of gp41 was found to inhibit virus induced cell fusion at concentrations of 0.1–1 μM (Slepushkin *et al.*, 1993). In the present study neither the fusion peptide of Sendai virus nor its analogues exhibited inhibitory effects at concentrations up to 4 μM (Figure 2). In the case of the HIV peptide, large aggregates were observed in aqueous solution and better results were obtained when the peptide was conjugated to carriers (Slepushkin *et al.*, 1993). One possible explanation of the lack of activity of the Sendai virus peptides is that massive aggregation of these hydrophobic peptides in the buffer made them incompetent for inhibition. Alternatively, the fusion domains may have different roles in the fusion mediated by the Sendai and HIV viruses. Two possible mechanisms have been suggested for the inhibitory effect of the HIV fusion peptide. The first is the presence of receptors or special places for interaction with the fusion region of gp41 on target cell membranes, and the second is that fusion peptides interact directly with gp41. The results of Freed *et al.* (1992), who showed that gp41 with a mutation in its fusion peptide region could dominantly block the fusogenic capacity of the native proteins, suggest a high-order interaction of envelope proteins and hence support the second proposed mechanism. Further support for the second mechanism is the observation that a synthetic peptide corresponding to the fusion peptide of Sendai virus was found to self-associate in its membrane bound state (Rapaport and Shai, 1994).

Heptad repeats as common structural domain in fusion proteins

It was reported that a synthetic peptide (DP-107) corresponding to a coiled-coil region of the gp41 of HIV-1 blocked virus-mediated cell–cell fusion in the micromolar range (Wild *et al.*, 1992). Another synthetic peptide (DP-178), modelled after a region of the gp41 adjacent to the transmembrane domain, was an even more potent inhibitor of virus induced cell–cell fusion (Wild *et al.*, 1994a). A correlation between the ability of DP-178 to associate with the coiled-coil structure (modelled by DP-107) and its ability to inhibit HIV-1 infection was suggested (Wild *et al.*, 1995). These results propose that the anti-viral activity of DP-178 is the result of its ability to interact with and disrupt the function of the gp41 leucine zipper in the process of virus entry. Jiang *et al.* (1993a,b) reported on another anti-viral peptide (designated SJ-2176) that shares partial sequence homology with DP-178. A different mechanism of inhibition mechanism was proposed for this peptide, namely that it interacts with the N-terminal fusion domain of gp41 (Jiang *et al.*, 1993a,b).

We have synthesized two peptides corresponding to the heptad repeats of the Sendai F protein. The first, SV-163, is from a domain similar to that of the HIV DP-107 but with a shorter length, and the second, SV-473, is from a domain adjacent to the transmembrane region, as the peptides DP-178 and SJ-2176 from HIV. Our results demonstrated that while SV-163 was devoid of inhibitory effects under the conditions tested, SV-473 was a highly potent inhibitor of the fusion of Sendai virus with human erythrocytes (Figures 3 and 4). These results resemble those found for HIV, since DP-107, which is located at a position similar to that of the SV-163 peptide, was several 1000-fold less active than DP-178 (Wild *et al.*, 1994a), which is also adjacent to the transmembrane anchor of HIV, as is SV-473 in Sendai virus.

The mechanism of inhibition

The fusion process is a two-step reaction, involving a binding step and the actual fusion event. Similar to what has been found with HIV-derived anti-viral peptides (Wild *et al.*, 1994), the SV-473 peptide also did not interfere with the attachment of the virus to the target cell. Inhibition

was observed when the peptide was added to virus that had already been attached to the cells in the cold (Figure 3). In principle, peptides can interact either with the virus or with the cell plasma membrane. The results demonstrate that the SV-473 peptide can interact with both, however membrane insertion is not a general property for all amphipathic coiled-coils as SV-163 did not interact with membranes under similar conditions (Figures 5 and 7). It was also reported that a coiled-coil peptide from the GCN4 leucine zipper protein does not interact with membranes (O'shea *et al.*, 1991). Furthermore, membrane affinity is not sufficient for inhibitory potential, since the fusion peptide of Sendai virus and its G12A analogue have a high affinity for lipid vesicles (Rapaport and Shai, 1994), but do not possess inhibitory capacity (Figure 2).

Membrane affinity would create a high local concentration of the peptide in the proximity of its putative target. The surface location of the SV-473 peptide (Figures 5 and 6) might suggest that in the actual fusion process the peptide-membrane interaction is only transient and serves as a relay system to bring this domain into the proximity of a proteinous element. The data suggest that an interaction with viral component does occur (Figure 7).

It seems likely that the F protein undergoes a conformational change at the right time and in the right place, and this change would expose the fusion peptide (Lamb, 1993). Such conformational change-dependent exposure of the fusion peptide was observed in the influenza virus as part of the fusion triggering event (Carr and Kim, 1993; Bullough *et al.*, 1994). It is not clear whether the proposed interactions between functional domains that were suggested to be part of the inhibition mechanism of peptides derived from HIV-1 take place in the native or fusogenic state of the fusion proteins. It was suggested that the inhibitory potential of the HIV-1 DP-178 peptide stems from its ability to interact and disrupt the leucine zipper domain in proximity to the fusion domain (Wild *et al.*, 1995), preventing the latter from taking part in the sequence of events associated with viral entry (Wild *et al.*, 1994b). A peptide with a partially overlapping sequence to DP-178 was also found to be a potent inhibitor. However, an interaction with the fusion domain was proposed as the inhibition mechanism for this peptide (Jiang *et al.*, 1993a,b).

We suggest an additional possibility, namely that SV-473-like peptides interact with their respective domains on the intact virus and thus interfere with the correct assembly of the high-order fusogenic form of the fusion proteins. A domain adjacent to the transmembrane region was found to be involved in assembly of the trimer of the influenza HA_2 protein (Carr and Kim, 1993; Bullough *et al.*, 1994). Our results about the oligomerization of SV-473 in solution may support this proposal. Our suggestion can explain the fact that the SV-473 peptide and its HIV-1 equivalent (DP-178) are very potent even at a low concentration, and are much more potent than the peptides modelled after the leucine zipper domain (SV-163 and DP-107). The participation of even one synthetic peptide molecule in oligomeric structures needed for fusion could result in dominant interference leading to the inactivation of the entire complex. As it was found that the ability of DP-107 to form oligomers correlates with a role in virus induced fusion rather than assembly of the glycoprotein complex (Wild *et al.*, 1994b), this dominant interference is not expected from SV-163 or DP-107.

In conclusion, the results of the present study demonstrate that a synthetic peptide corresponding to a functional domain of the Sendai virus fusion protein exhibited a capacity to inhibit viral infection similar to that of peptides corresponding to parallel domains in HIV-1 gp41. Although the mechanism of anti-viral activity of these peptides remains to be elucidated, the similarity between the behaviour of the peptides from the two viruses indicates common steps in the fusion mechanism and may suggest that inhibition by synthetic peptides modelled after structural domains can be extrapolated to other fusogenic viruses.

Materials and methods

Materials

Butyloxycarbonyl amino acid phenylacetamidomethyl resins were purchased from Applied Biosystems, and amino acids were obtained from Peninsula Laboratories. NBD–fluoride and other reagents for peptide synthesis were bought from Sigma. PC, PE and PS from bovine spinal cord (sodium salt grade I) were purchased from Lipid Products. The probe 5-(and 6-)carboxytetramethyl-rhodamine, succinimidyl ester was purchased from Molecular Probes. Proteinase K (fungal), chromatographically purified and lyophilized, was purchased from Merck.

Synthesis, fluorescent labelling and purification of peptides

The peptides were synthesized by a solid phase method as previously described (Shai *et al.*, 1990). Labelling of the N-terminus of the peptides was achieved as previously described (Rapaport and Shai, 1991); briefly, resin-bound peptides were treated with trifluoroacetic acid, followed by addition of the fluorescent probe and finally cleaved from the resins by HF. The synthetic peptides were purified (>95% homogeneity) by reverse-phase HPLC on a C_4 column using a linear gradient of 25–80% acetonitrile in 0.1% TFA, in 40 min. The peptides were subjected to amino acid analysis in order to confirm their composition.

Virus and erythrocytes

Sendai virus (Z strain) was grown in the allantoic sac of 10–11-day old embryonated chicken eggs, harvested 48 h after injection and purified according to Peretz *et al.* (1974). The virus was resuspended in buffer composed of 160 mM NaCl, 20 mM tricine, pH 7.4, and stored at –70°C. Virus haemagglutinating activity was measured in haemagglutinating units (HAU) as described (Peretz *et al.*, 1974). One microlitre contained ~60 000 HAU (Borkow and Ovadia, 1992).

Fresh human blood was obtained from a blood bank and stored for up to 1 month at 4°C. Prior to use, erythrocytes were washed twice with phosphate buffered saline (PBS), pH 7.2, and diluted to the desired concentration (% v/v) with the same buffer.

Interaction of Sendai virions with human erythrocytes (haemolysis assay) and determination of anti-viral activity of the peptides

Virions, erythrocytes and peptides were mixed in different orders of addition and various amounts. The final incubation was always at 37°C for 30 or 60 min, followed by centrifugation at 5700 *g* for 10 min to remove intact cells. In all cases duplicate samples were used and two aliquots were taken from the supernatant of each sample to two wells of a 96-well plate. The amount of haemoglobin release was monitored by measuring the absorbance of the wells with an ELISA plate reader at 540 nm.

Preparation of lipid vesicles

Dry lipids were hydrated in buffer and dispersed by vortexing to produce large multilamellar vesicles. Small unilamellar vesicles (SUV) were then prepared by sonication, while large unilamellar vesicles (LUV) were prepared by first freeze–thawing five times and then extruding 10 times through two polycarbonate membranes with 0.1 μm pore diameter (Hope *et al.*, 1985). The lipid concentrations of the liposome suspensions were determined by phosphorus analysis (Rouser *et al.*, 1966).

NBD fluorescence measurements

Changes in the fluorescence of NBD-labelled peptides in buffer were measured following the addition of virions or vesicles to their solution. Emission spectra were recorded with the excitation set at 465 nm (10 nm slit). The time profiles were recorded with the excitation set at 465 nm (10 nm slit) and the emission at 530 nm (8 nm slit). All fluorescence measurements were done at 37°C using a magnetic stirrer on a Perkin-Elmer LS-50B Spectrofluorometer.

Enzymatic digestion of membrane-bound SV-473

The accessibility of NBD–SV-473 to proteolytic digestion when bound to lipid vesicles was investigated. Lipid vesicles composed of PS/PE/PC (2.8/1.5/1 molar ratio) were added to a labelled peptide (0.55 μM, in 150 mM NaCl, 10 mM sodium phosphate, pH 7.3 at 37°C) followed by the addition of 4 μl of proteinase K solution (0.25 mg/ml, in the same buffer). The fluorescence intensity was monitored at 530 nm (8 nm slit) with the excitation set at 465 nm (10 nm slit). In a control experiment proteinase K was added first to the peptide solution followed by the addition of liposomes.

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