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Supporting Material

A Helical Conformation of the SEVI Precursor Peptide PAP248-286, a Dramatic Enhancer of HIV Infectivity, Promotes Lipid Aggregation and Fusion

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Materials and methods

Materials

The $PAP_{248-286}$ peptide was synthesized by Biomatik (Toronto, ON). Lipids (POPG (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol)), POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine), POPS (1-palmitoyl-2-Oleoyl-*sn*-Glycero-3-[Phospho-L-Serine]), POPE (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine), sphingomyelin (porcine brain), and cholesterol were acquired from Avanti (Alabaster, AL). HFIP (hexafluoroisopropanol) DMSO (dimethyl sulfoxide), TFA (trifluoroacetic acid), and carboxyfluorescein were obtained from Sigma-Aldrich. Polybrene (Hexadimethrine bromide) and polylysine (mol. wt. 1000-4000) were purchased from Sigma.

Sample Preparation

SEVI refers to the fibrillar form of a series of peptides derived from PAP that form amyloid fibers in solution and increase the infectivity of the HIV virus. A variety of peptides within the 247-286 sequence of PAP are active, with a stringent requirement for the LIMY sequence (residues 283-286) at the C-terminus. We have chosen $PAP_{248-286}$ for this study as it is the most effective form of SEVI at enhancing HIV infectivity (sequence shown in Fig. 1). Because preformed aggregates of the peptide can act as nuclei for further peptide aggregation and lead to inconsistent results, it is essential that the peptide be completely dissolved initially in a solvent that strongly disfavors further aggregation. Initial attempts to solubilize the peptide at a concentration of 1 mg/ml by hexafluorisopropanol (HFIP), a solvent commonly used for this purpose for other amyloid proteins, failed. However, the peptide was soluble in a 1:1 solution of trifluoroacetic acid and HFIP at a concentration of 5 mg/ml and this method was used for the initial solubilization of the peptide in subsequent experiments. TFA and HFIP were removed from the sample by a stepwise procedure. The TFA/HFIP solvent mixture was first evaporated by a nitrogen stream and the subsequent peptide film was redissolved in pure HFIP at a concentration of 5 mg/mL. HFIP was then removed by lyophilization overnight (approximately 12 hours) at a 1 mTorr vacuum. The lyophilized peptide was then redissolved in either DMSO for dye leakage studies or buffer (50 mM sodium phosphate with 150 mM NaCl) at a concentration of 100 μM and used immediately. Human-IAPP was prepared identically to $PAP_{248-286}$ and used immediately. Polylysine and polybrene, which do not aggregate and are highly soluble in water, were directly prepared in buffer.

Four types of model membranes were used in this study. The first, 7:3 POPC/POPG, is a simple model membrane commonly used to simulate a mixed anionic/zwitterionic membrane system. POPG vesicles were used in the dye leakage assays. A vesicle composition of 39.2% POPC, 18.5% POPE, 11.5% POPS, 7.7% Sphingomyelin and 23% Cholesterol was used to model the membrane of a typical host cell. (18) A typical viral cell membrane composition was modeled using 22% POPC, 17.3% POPE, 10% POPS, 17.3% Sphingomyelin and 33.3% Cholesterol.(18) Vesicles of 100 nm diameter for liposome aggregation and dye leakage studies were prepared by extrusion as previously described.(19) Small unilamellar vesicles (SUVs) were prepared by bath sonication of a 1 mg/ml solution of lipids for two periods of 10 minutes each.

Liposome Aggregation

The ability of human-IAPP, polybrene, polylysine, and $PAP_{248-286}$ to aggregate lipid vesicles was measured by the increase in turbidity at 350 nm immediately after the addition of a freshly prepared $PAP_{248-286}$ on a microplate recorder using clear polystyrene plates.

Dye Leakage Assay.

For the dye leakage experiments, carboxyfluorescein-containing POPG vesicles were prepared by rehydrating the dried lipid film in 50 mM sodium phosphate buffer (pH 7.5) containing 40 mM carboxyfluorescein, adjusted to pH 7.5 by the addition of sodium hydroxide.

Nonencapsulated carboxyfluorescein was removed from the vesicles through size exclusion chromatography using a PD-10 column (Amersham Pharmacia Biotech, Uppsala, Sweden). Vesicle solutions were used immediately, and a fresh vesicle solution was used for each experiment. Fluorescence readings were taken at an excitation wavelength of 493 nm and an emission wavelength of 518 nm using 2 nm excitation and emission slit widths. A baseline reading was taken on the solutions prior to the addition of the peptide. After injection, the fluorescence intensity was recorded after 100 s of interaction. The fluorescence signal given by the addition of peptide was then normalized by the addition of Triton X detergent, causing all vesicles present to release any remaining dye to obtain the total possible fluorescent signal.

Differential Scanning Calorimetry

Peptide stock solutions in methanol (4 mg/ml) were prepared from lyophilized peptide treated as described above. The stock solution was then added to the lipid (either DiPoPE or 7:3 DMPC/DMPG) in choloroform and the solution first dried under a stream of nitrogen and then further dried under high vacuum for several hours to remove residual solvent. Buffer (10 mM Tris/HCl, 100 mM NaCl, 1 mM EDTA, 0.002 % w/v NaN_3, pH 7.4 for the DiPOPE sample and 10 mM Sodium Phosphate, 150 mM NaCl, pH 7.3 for the DMPC/DMPG sample) was added to the film to produce 4 mg/ml solutions. The DMPC/DMPG samples were subjected to 5 freeze/thaw cycles before use. Changes in C_p was recorded from 10- 60°C with a heating rate of 1°C/min for the DiPOPE samples and 5-60°C with a heating rate of 0.5°C/min for the DMPC/DMPG samples.

Circular dichroism spectroscopy

CD samples were prepared by dissolving lyophilized peptide to a concentration of 14 μM in sodium phosphate buffer (10 mM with 150 mM NaF, pH 7.3) and briefly vortexing and sonicating (approximately 15 seconds) before transferring to a 0.1 cm cuvette. After the initial spectrum of $PAP_{248-286}$ in the absence of lipids was taken, POPE/POPG vesicles from a 40 mg/ml stock solution were titrated into the cuvette. Spectra were measured at 1 nm intervals from 185 nm to 260 nm at a scanning speed of 50 nm/min and a bandwidth of 1 nm. Each spectrum reported is the average of ten scans after subtraction of the baseline spectrum (buffer and vesicles without peptide).

Membrane Fusion

Membrane fusion was followed by a lipid mixing assay based on the decrease in fluorescent resonance energy transfer (FRET) when labeled liposomes containing both a donor and acceptor pair fuse with unlabelled liposomes. Fusion of the labeled liposomes with unlabeled liposomes increases the average distance between the donor and acceptor lipids and therefore decreases the FRET efficiency. Small unilamellar vesicles (SUVs) containing 68% POPC, 30% POPG, and 1% NBD-PE and 1% Rhodamine-PE were prepared by sonication and mixed with a 20x fold excess of unlabelled SUVs. FRET was detected from the rhodamine emission peak at 590 nm using excitation of NBD at 440 nm. Samples were run on polystyrene microplates on a Synergy 2 microplate reader using deep blocking filters with an excitation bandwidth of 30 nm and emission bandwidth of 35 nm.