Computational Evaluation of Mel4 and Lactoferricin Interactions with Adenovirus and Norovirus Capsids

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Introduction

- Human adenovirus and norovirus are environmentally stable, non-enveloped viruses that remain major causes of infections worldwide.
- Effective antiviral therapeutics remain limited.
- We have developed a novel antimicrobial peptide, Mel4[1]
- Mel4 and lactoferricin (LFc) possess antiviral activity against adenovirus type 5 (HAdV-5) and murine norovirus type 1 (MNV-1)[2]
- At active concentrations they are not toxic to the host mammalian cells.[2]

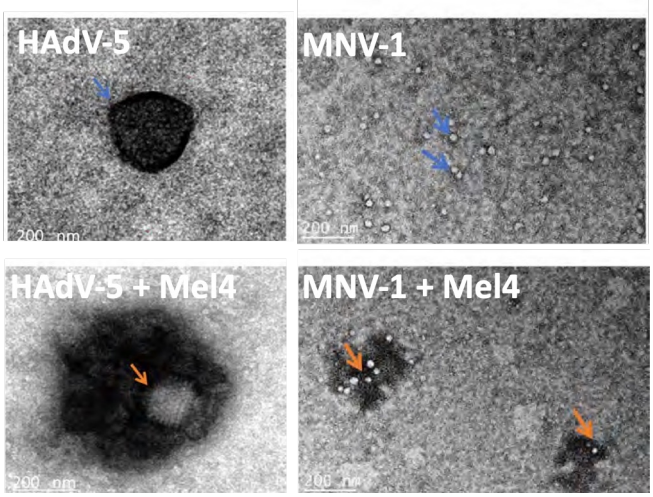
AIM

This study aims to determine the mechanism of action of Mel4 and LFc using electron microscopy and in silico approaches.

Materials and Methods

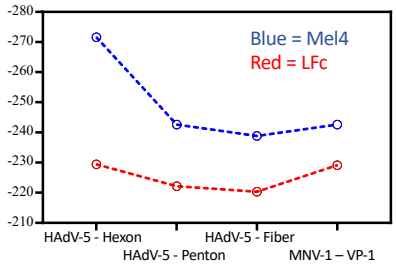
- Human Adenovirus type 5 (HAdV-5; ATCC-VR-5) and murine norovirus type 1 (MNV-1; ATCC-VR-1937)
- Mel4 (KNKRKRRRRRRGGRRRR) and LFc (RRWQWRMKKLE)
- Viruses exposed to the peptide/mimics for 2 h at 37 ◦C. Stained with 2% phosphotungstic acid. Examined using transmission electron microscopy.
- For HAdV-5, the hexon, penton, and fiber proteins were obtained, while for MNV-1, the capsid protein VP1 was included.
- Structural models were predicted using AlphaFold3
  - Structures were visualized and examined using PyMOL (Schrodinger; https://www.pymol.org) and Discovery Studio.
- Docking used HDOCK (http://hdock.phys.hust.edu.cn/)
- GROMACS software package was used to perform molecular dynamics simulations on a katana GPU-enabled platform.
- The particle mesh Ewald (PME) approach was used to determine long-range electrostatic interactions, and a cutoff distance of 1.2 nm was used for van der Waals interactions.

RESULTS:



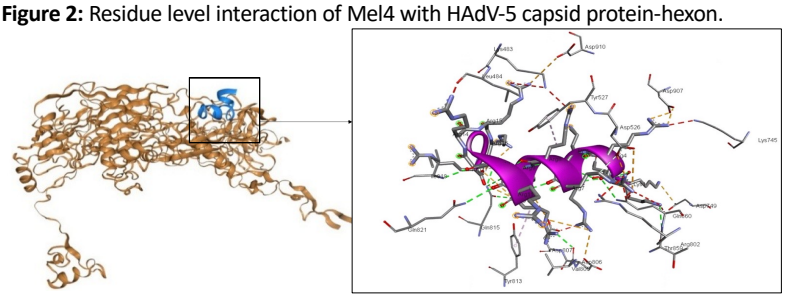
- HAdV-5, Mel4 had IC50 of 47.4 µM but LFc had no activity. Mel4 targeted the capsid, releasing electron dense material
- MNV-1, Mel4 had IC50 of 8.6 µM, LFc had IC50 of 23.18 µM. Mel4 also resulted in release of electron dense material

Figure 1: Comparative docking score of Mel4 and LFc against HAdV-5 and MNV-1 capsid proteins.



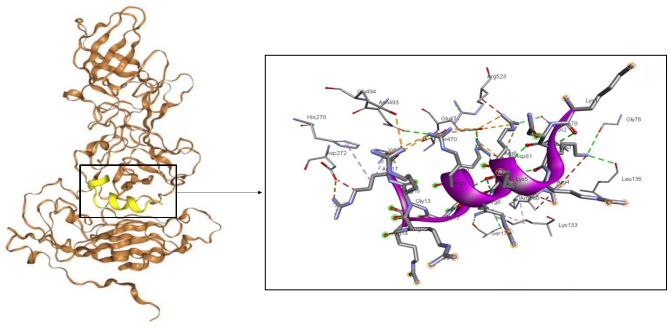
Mel4 had a stronger binding affinity toward key capsid proteins of both adenovirus and MNV-1 compared to LFc

Figure 2: Residue level interaction of Mel4 with HAdV-5 capsid protein-hexon.



- Mel4 formed contacts with residues Lys483, Leu484, Asp526, and Tyr527, positioned near the interface between the neck and tower domains.
  - Asp526 and Tyr527 lie firmly within the tower domain, which contribute to the surface-exposed loops of the hexon.
- Additional binding residues, Lys745 and Asp749, were identified within the tower domain near its C-terminal region.
- Further interactions were detected with residues Thr859, Gln860, Asp907, and Asp910, a distal region likely representing flexible or tail extensions of the hexon.
- Mel4 also engaged with Arg802, Val805, Asp806, Asp807, Tyr813, Gln815, Ile818, and Gln821, within the core domain of the hexon, suggesting stabilization across surface and internal regions.

Figure 3: Residue level interaction of Mel4 with MNV-1 capsid protein-VP-1.



- Mel4 bound to the major capsid protein VP1 of MNV-1 near the P1 region
  - Specifically, residues Gly76, Glu77, Ile78, and Asp81, located in the N-terminal domain, which are involved in capsid formation and protomer interfaces.
- Additional contacts were observed with Lys133, Leu136, Ser178, Arg180, His270, Asp272, Val468, Ser470, Glu471, Asn493, Gly494, and Arg523, all of which were situated within the shell domain.
- These interactions suggested that Mel4 associated with both structural domains critical for capsid stability and assembly.

Table 1: Molecular dynamics of Mel4 and Lactoferricin complexes with viral proteins

Peptide	Proteins	Root Mean Square Deviation (RMSD) (nm)*	Radius of Gyration (Rg) (nm)*	Solvent Accessible Surface Area (SASA) (nm^2) *
Mel4	Hexon	~1.3	~3.5	~480-500
	Penton	~0.3-0.4	~3.02-3.08	~245-265
	Fiber	~0.25-0.35	~1.63-1.70	~100-115
	VP-1	~0.4	~2.90-2.92	~285-287
Lactoferricin	Hexon	~1.7	~3.8-3.9	~440-470
	Penton	~0.45-0.6	~3.12-3.13	~260-280
	Fiber	~0.35-0.55	~1.70-1.90	~110-130
	VP-1	~0.9 nm	~3.02	~278-280

*Higher RMSD = less structural stability (more deviation from initial structure); Higher Rg = less compact structure (more expanded); Higher SASA = more surface exposed to solvent (greater interaction potential)

References:

1. Willcox MDP, Chen R, Kalaiselvan P, Yasir M, Rasul R, Kumar N, Dutta D. The development of an antimicrobial contact lens – from the laboratory to the clinic. Current Protein & Peptide Science. 2020; 21: 357-368
2. Urmi UL, Vijay AK, Willcox MDP, Attard S, Enninful G, Kumar N, Islam S, Kuppusamy R. Exploring the efficacy of peptides and mimics against influenza A virus, adenovirus, and murine norovirus. Int J Mol Sci. 2024; 25: 7030