Optimization of a TREM2 receptor inhibitor peptide

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Introduction

Triggering receptor expressed on myeloid cells 2 (TREM2) is an immune receptor regulating the inflammatory response and involved in neurodegenerative, chronic and autoimmune diseases. TREM2 is an orphan receptor, and the research of new ligands has recently become a hot topic. In this context, the peptide sequence 1A9 (H-IFLIKILAA-OH) has been rationally designed for inhibition of TREM2 [1]. In order to deeper analyze the mechanism of interaction with TREM2 and select molecules with specific binding, we synthetized a series of 1A9 peptide analogues where the **Lys** residues was replaced by either **Gly** or **Ala** to explore the effect of the residue side chain charge and polarity in this position that is believed to be fundamental for ligand interaction. Their conformation, their ability to interact with membranes, and their biological activity was investigated.

Synthesis and purification

Peptides were synthesized by Fmoc-SPPS starting from a 2-chlorotrytil resin. The lipophilic nature of the sequences required 5% piperazine + 2% DBU for Fmoc removal, in order to suppress aggregation during chain elongation, and HATU as coupling reagent.

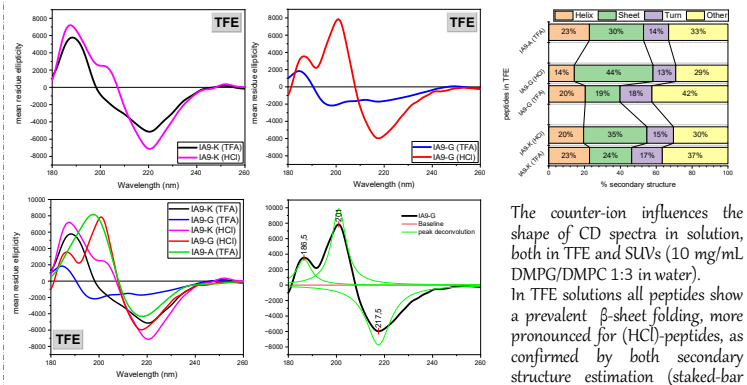
RP-HPLC purification was challenged by the low solubility of peptides in conventional solvents and buffers but was overcome dissolving the peptides in either DMSO or HFIP (1,1,1,3,3,3-Hexafluoro-2-propanol).

In order to investigate the interaction of peptides with membranes as a function of counterion, the RP-HPLC purified peptides were repeatedly dissolved in diluted hydrochloric acid solution and freeze-dryed in order to exchange TFA with HCl.

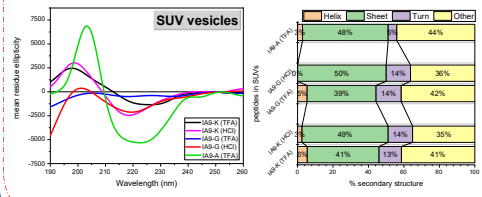
Conformational characterization

Circular Dichroism in solution

The secondary structure of a peptide in a membrane environment can be characterized using conventional CD in aqueous dispersions of small unilamellar vesicles or exploiting TFE as membrane-mimetic solvent.



The counter-ion influences the shape of CD spectra in solution, both in TFE and SUVs (10 mg/mL DMPG/DMPC 1:3 in water).
In TFE solutions all peptides show a prevalent β-sheet folding, more pronounced for (HCl)-peptides, as confirmed by both secondary structure estimation (staked-bar plot) and signal deconvolution (as an example 1A9-G(HCl)), where an intense negative band centered at ~217 nm and a positive signal at 187 nm is detected. However, (HCl)-peptides show two positive signals, one centered around at 186 nm and one at 201 nm, the first more intense in 1A9-K, the second in 1A9-G.
1A9-G (TFA) shows a positive band at ~186 nm and two negative bands at 198 and 217 nm, suggesting an equilibrium between unordered and β-sheet folding due to the proximity of the respective dihedral angles. 1A9-A instead shows a single positive signal centered at 198 nm, and a negative one at 222 nm, suggesting a β-stranded structure, with the negative band red-shifted compared to the canonical value of 217 nm, suggesting a possible antiparallel conformation [2].

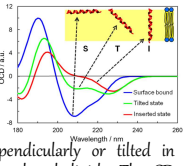


In SUVs 1A9 peptides shows a more prevalent β-sheet folding compared to TFE solutions, as confirmed by secondary structure estimation (staked bar plot). All spectra show a negative band, progressively red-shifted in the series 1A9-K(HCl) > 1A9-G(HCl) > 1A9-K(TFA)> 1A9-G(TFA) > 1A9-A(TFA), suggesting a prevalently antiparalel β-conformation [2].

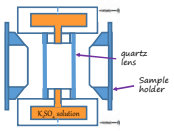
Oriented Circular Dichroism (OCD)

OCD is used to evaluate the conformation and orientation of membrane-active peptides in oriented membrane systems.

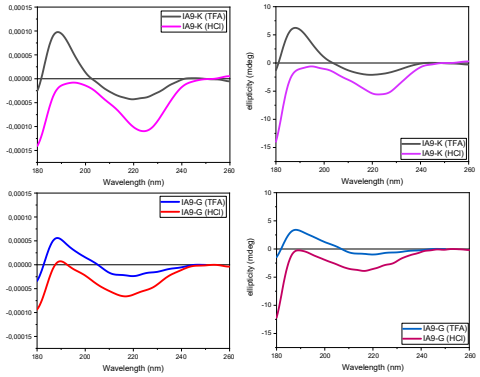
The most studied peptides in this fiels are mainly folded in α-helical conformation, and show different OCD spectra when bound to the surface or when inserted perpendicularly or tilted in between the membrane phospholipids. The CD signal indeed depends on the angle between the helix axis and the electric field vector of the circularly polarized light beam, which is perpendicular to the direction of propagation [3]. Little data is reported for β-sheet folded peptides, only comparing the OCD signal to the CD spectral features observed in solution.



20μg of peptide were mixed with 500μg of DMPG/DMPC 1:3 dissolved in MeOH/$CHCl_3$ 1:1 v/v, deposited on quartz windows and dried for 24h under vacuum. The sample is humidified via the gas phase derived from saturated K_2SO_4 solution, which achieves a relative humidity of ~98%.
The same humidity is ensured by the presence of the K_2SO_4 solution in the cell's reservoir.

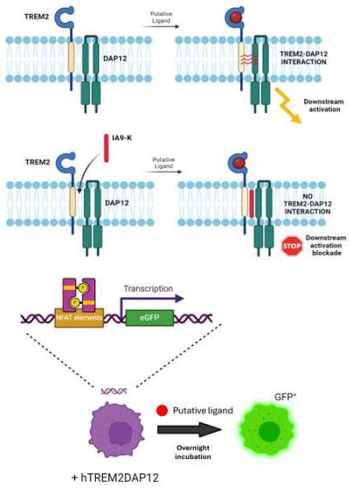


Custom-built cell



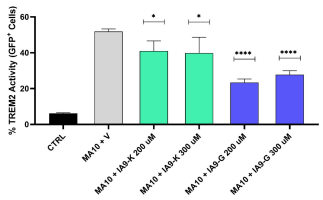
Spectra are reported in g-factor (ellipticity over absorbance) to account for the different thickness of each sample.
The different counterions seem to affect the interaction of the peptides with the membranes.
(TFA)-peptides show a positive band at ~190 nm and a broad negative one at ~220 nm, while in (HCl)-peptides the intensity of the positive band is diminished, and the negative one increases and is red-shifted.

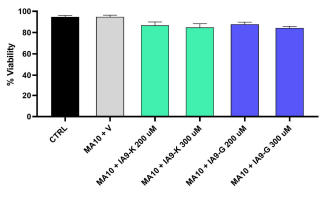
Biological activity



The signaling chain homooligomerization (SCHOOL) strategy aims to block multi-chain receptors in a ligand-independent manner using short peptides [4].
In the figure is reported the scheme of SCHOOL strategy for TREM2. Briefly, a competition for DAP12 binding occurs between the short peptide and the transmembrane portion of TREM2.
IA9-K with DAP12 interaction prevents message transduction in the presence of the putative ligand, causing blockade of downstream signaling. This mechanism appears to have a maximum effectiveness of 50% reduction.

2B4 reporter T cells stably transfected with enhanced green fluorescent protein (eGFP) under consensus sequences of NFAT (transcriptional factor of TREM2 pathway) and human TREM2/DAP12 cDNA has been employed to assay the inhibitory capacity of the synthetic peptides.





Statistical analysis Ordinary one-way ANOVA (*p ≤ 0.05, **** p ≤ 0.0001 versus MA10 + V), n=2

Different solvent conditions and concentrations have been tested to obtain the optimal result. 1A9-K (TFA) and 1A9-G (TFA) have been dissolved in DMSO/PBS (1:1 v/v) and tested at 200 μM and 300 μM.
Mycolic Acid, a known TREM2 putative ligand [5], has been used to induce receptor activation measured as eGFP+ positive cells by FACS at the concentration of 10 μg/mL (MA10) after overnight incubation.
In this model both 1A9-K and 1A9-G have been able to reduce TREM2 acivity significantly, in particular 1A9-G has shown greater inhibitory effectiveness by reducing the GFP+ population of the 50% compared to vehicle control (MA10 + V). These results are in line with data reported in the literature regarding the *in vivo* experiments.

The cell viability evaluation excluded potential toxic effect induced by peptides treatment. Propidium iodide dye has been used for flowcytometric analysis. No differences have been measured in presence of 1A9-K and 1A9-G compared to CTRL (vehicle).

Future perspectives

CD spectra of 1A9 peptides, adopting a prevalently β-sheet folding in different membrane-like environment, revealed the influence of the counter-ion on peptide conformation. Future investigation will focus on the effect of the different counter-ions on the ability of the peptides to interact with the receptor and their influence on the biological activity.

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