ACTIVITY AND SELECTIVITY OF PGLa-H TANDEM REPEAT PEPTIDES AGAINST MULTIDRUG RESISTANT CLINICAL BACTERIAL ISOLATES

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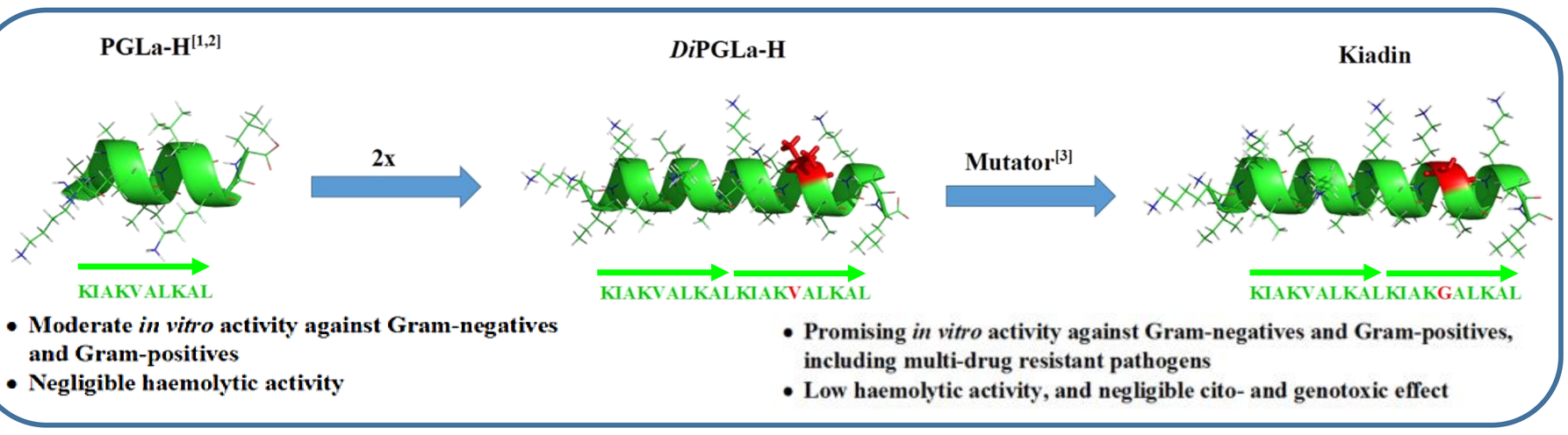
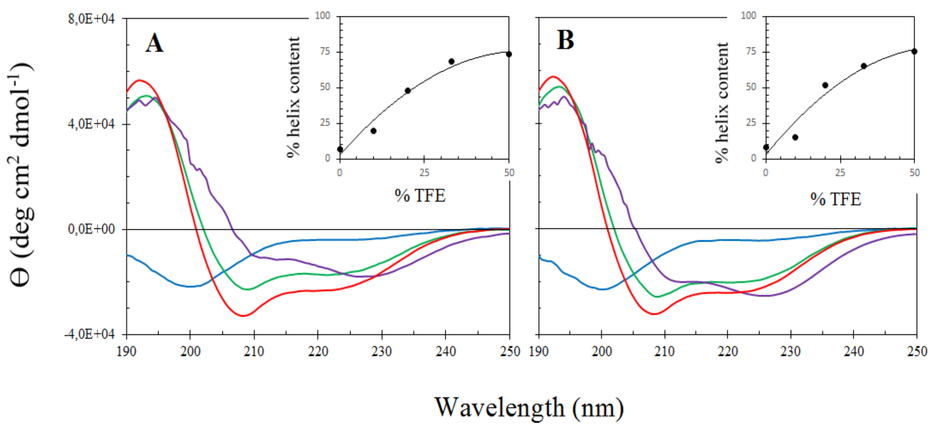
CONCEPT



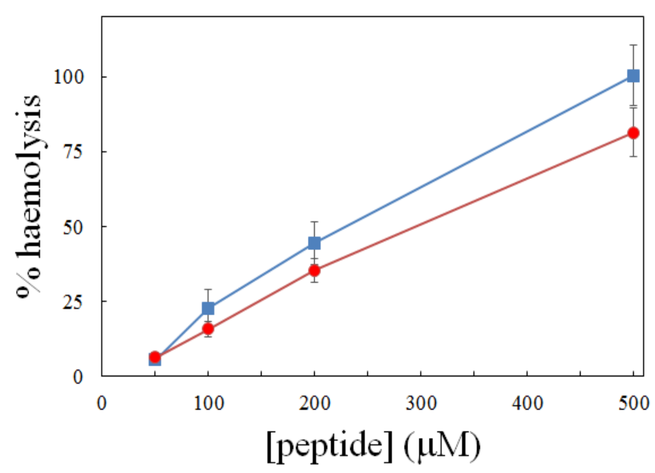
RESULTS

STRUCTURE:



CD Spectra of *di*PGLa-H (A) and kiadin (B). Both peptides (20 μM) are random coil in aqueous buffer (—), but α helical in 10 mM SDS (—) or 50% TFE (—) (see inset). Spectral shape with anionic LUVs (PG:dPG 95:5, 0.4 mM phospholipid, —) suggests aggregation.

HAEMOLYSIS:



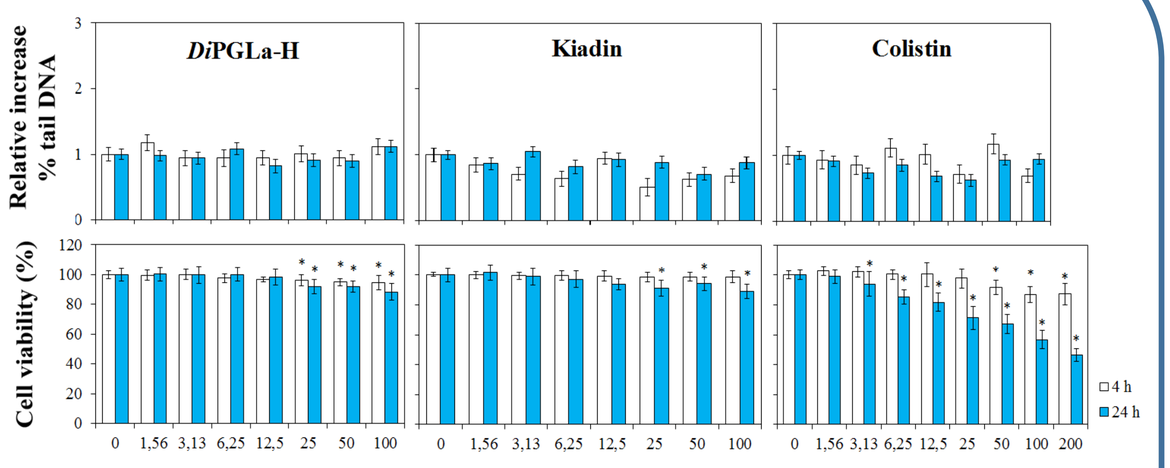
Assays on human RBC indicate low toxicity for both peptides with HC_{50} values estimated at 270 ± 30 μM for *di*PGLa-H (—) and 340 ± 30 μM for kiadin (—).

ANTIMICROBIAL ACTIVITY:

MIC and MBC values vs laboatory strains and drug resistant clinical isolates and relative selectivity indices (SI = HC_{50}/MIC).

	*Di*PGLa-H			Kiadin		
	MIC	**MBC**	**SI**	**MIC**	**MBC**	**SI**
Gram-negative						
E. coli ATCC 25922	1.5	1.5	180±20	0.75	0.75	450±40
E. coli c.i.	6	12	45±5	12	24	30±5
K. pneumoniae ATCC 13883	3	3	90±10	3	3	115±10
K. pneumoniae c.i.	12	24	22.5±2.5	12	24	30±5
A. baumannii ATCC 19606	1.5	1.5-3	180±20	1.5	1.5	225±20
A. baumannii c.i.	1.5-3	3	135±50	1.5	1.5	225±20
P. aeruginosa ATCC 27853	6	12	45±5	6	12	60±5
P. aeruginosa c.i.	6	12	45±5	3	6	115±10
Gram-positive						
S. aureus ATCC 29213	0.75	1.5	360±40	0.5-1	1.5	450±40
S. aureus c.i.	1.5	1.5	180±20	3	3	115±10

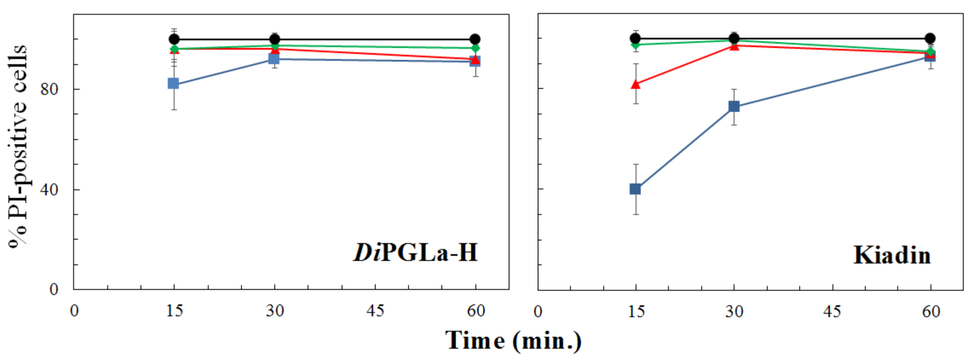
HPBL VIABILITY & DNA DAMAGE:



Effect of *di*PGLa-H and kiadin after exposure of human peripheral blood leukocytes, HPBLs for 4 and 24 h. Effect on viability assessed by differential staining with AO/EtBr (bottom panels). DNA damage as % DNA in comet assay tail (upper panel). **Low cytotoxicity and no DNA damage.**

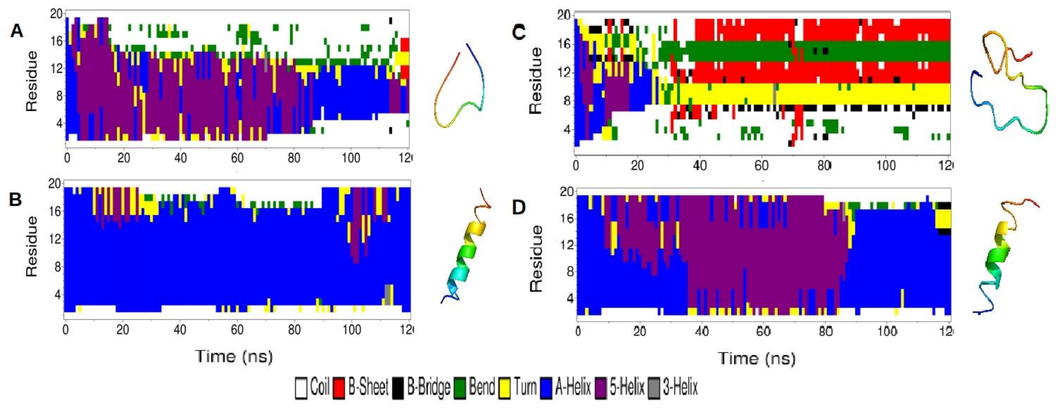
*Statistically significant compared to corresponding control (P<0.05).

MEMBRANE PERMEABILIZATION:



Bacterial membrane integrity measured by flow cytometry on *E.coli* ATCC 25922 cells, exposed to 0,5 (—), 1 (—) and 2 (—) μM peptide concentrations. Fast permeabilization is typical for membranolytic AMPs, and suggests a similiar mode of action for both peptides. Melittin (5 μM, —) was used as positive control.

STRUCTURE MODELLING:



Secondary structure of *di*PGLa-H and kiadin as a function of simulation time in H_2O and TFE/H_2O solution. Simulation using Gromacs 4.6.5 package. A) *di*PGLa-H in water; B) *di*PGLa-H in TFE; C) kiadin in water; and D) kiadin in TFE. The structure on the right corresponds to the last conformation of the peptide after 120 ns in the corresponding solution.

CONCLUSIONS

- Doubling the size of a small but poorly active AMP significantly increased its potency without affecting selectivity.
- CD studies indicate that both longer AMPs are random coil structure in aqueous solution, but substantially helical in anisotropic environments.
- *Di*PGLa-H and Kiadin show potent and broad spectrum antibacterial activity (one Gram +, 4 Gram- species tested) also against MDR clinical isolates.
- These AMPS are potentially useful lead compounds due to reduced cytotoxicity *in vitro*, especially in comparison to the last resort antibiotic colistin.

Acknowledgements:

Authors acknowledge funding from Croatian Science Foundation project 8481 BioAmpMode. Department for Life Sciences (Trieste) acknowledges support from Beneficentia Stiftung, Lichtenstein.

References:

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