Membrane-bound structure of the short peptaibol Harzianin HK-VI

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Introduction

Peptaibols

- Natural membrane-active peptides isolated from fungi
- Abundant in Aib (U) (α-aminoisobutyric acid), possess a C-terminal alcohol and N-terminal acetylation/alkylation
- Display wide range of antimicrobial activties
- Able to lyse lipid membranes by pore formation

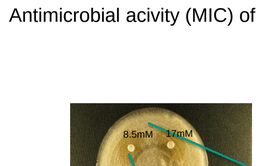
Harzianin HK-VI (HZ wt) is an ultra-short peptaibol (11-mer) isolated from *T. pseudokoningii* [1] with the sequence:

Ac-U-N-I-I-U-P-L-L-U-P-L-ol

Research aim

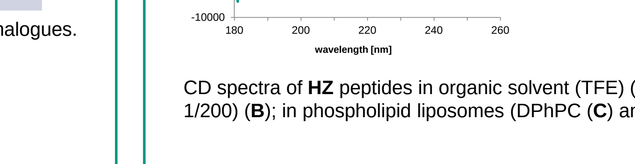
To solve the structure of membrane-bound **HZ** and get insights into its interactions with lipid bilayers.
How can the short **HZ** span the bilayer with the expected α-helical structure?

HZ as α-helix and 3_{10}-helix



Lipid	D_B [Å]*	$2D_C$ [Å]*
DLPC	32.6	21.7
DMPC	36.7	25.7
POPC	39.1	28.8
DPhPC	35.4	27.2

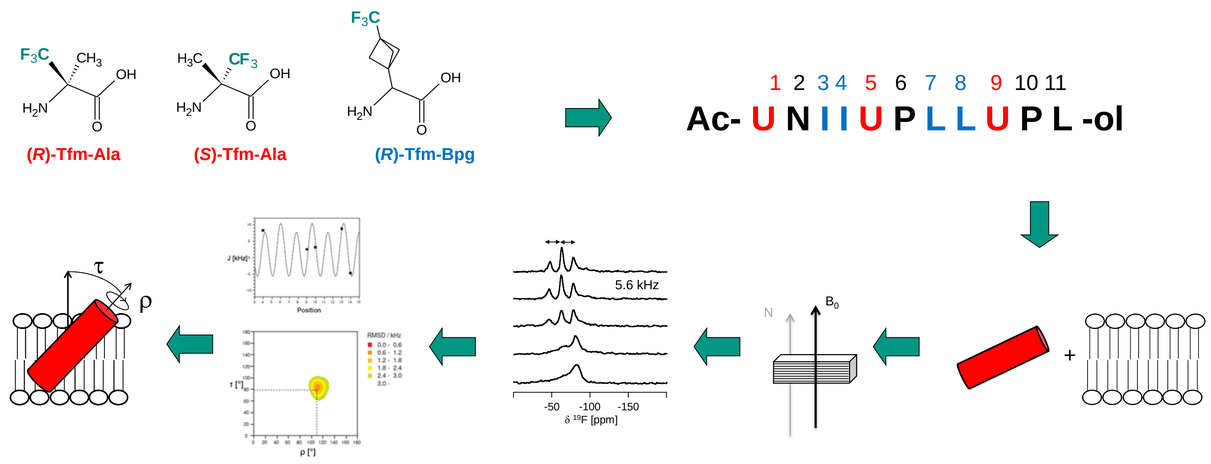
Inconsistency between peptide length and bilayer thickness.
D_B: bilayer thickness; $2D_C$: thickness of hydrocarbon region
*T=30°C [2]



Suggested mechanisms of peptide/bilayer interactions for short pore-forming peptides: arranging as double-layered channels (**1**); conformational change to extended conformations; (**2**) membrane thinning (**3**).

Methods

- **Solid-state ^{19}F-NMR** and **synchrotron circular dichroism (SRCD) spectroscopy** to determine the structure and alignment of **HZ wt** in lipid bilayers
- ^{19}F-NMR is enabled by one-at-a-time incorporation of synthetic α-trifluoromethylated amino acids: (*R*)-, (*S*)-Tfm-Ala [3,4] and (*R*)-Tfm-Bpg [5]
- Synthetic peptides are reconstituted in mechanically **aligned** (oriented) **lipid bilayers**
- From the ^{19}F-NMR dipolar couplings, the ***structure***, ***orientation*** and ***dynamics*** is determined [6]



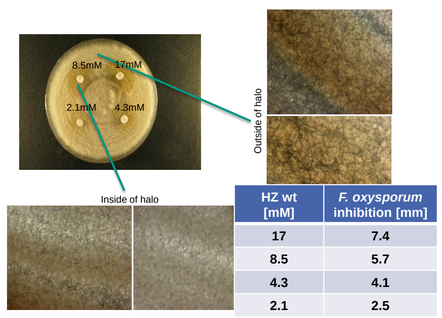
- Oriented SRCD from non-labelled peptide (**HZ wt**) with the synchrotron UV/VIS light source ANKA complements the results for the overall alignment of peptides

Results

Antimicrobial tests

	MIC [µg/ml]	
Bacteria	**HZ wt**	**HZ Tfm analogues**
E. coli K12	> 256	> 256
S. aureus DSM 1104	**128**	> 256
S. xylosus DSM 20267	> 256	> 256
E. faecalis DSM 2570	> 256	> 256
B. subtilis ATCC 6633	> 256	> 256
Fungi		
A. nidulans (GR5)	> 256	> 256
C. tropicalis	> 256	> 256
M. oryzea (Guy 11)	**32**	**32**
T. harzianum	> 256	> 256

Antimicrobial acivity (MIC) of **HZ wt** and ^{19}F-labeled analogues.

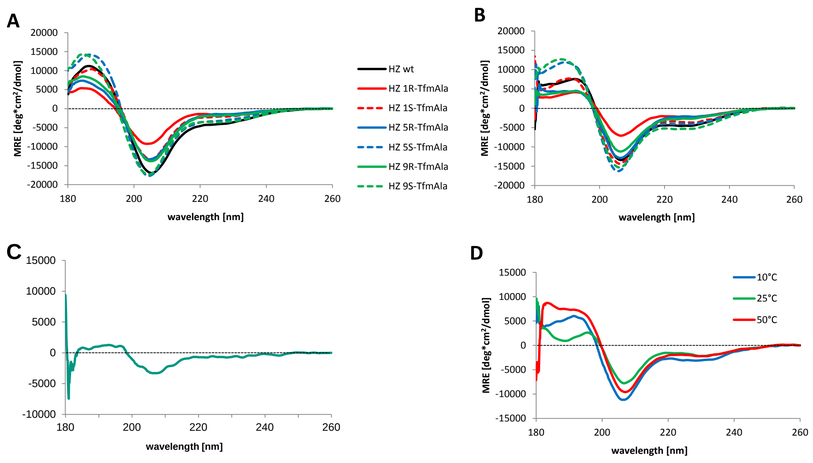


HZ wt [mM]	*F. oxysporum* inhibition [mm]
17	7.4
8.5	5.7
4.3	4.1
2.1	2.5

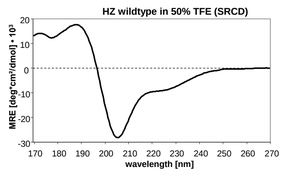
Plate diffusion assay testing **HZ wt** against *F. oxysporum.*

No pronounced antibacterial activity
Low antifungal effect against selected plant pathogenic fungi

Structure determination by CD



CD spectra of **HZ** peptides in organic solvent (TFE) (**A**); in detergent micelles (DPC, P/D 1/200) (**B**); in phospholipid liposomes (DPhPC (**C**) and DMPC (**D**), P/L 1/20).

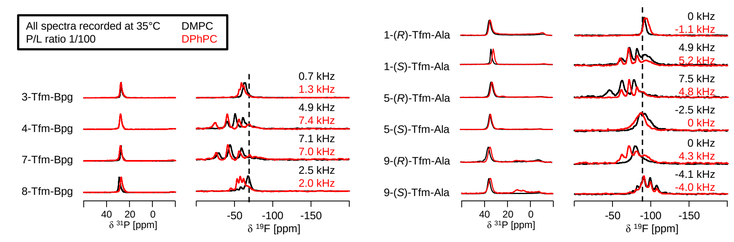


Algorithm	α	3_{10}	β	turns	PPII	NRMSD
CONTIN	**31.3**	**10.4**	0	14	9.8	0.253
SELCON3	**29.8**	**10.2**	-2.3	13.5	8.8	0.132
CDSSTR	**39**	**10**	5	11	9	0.005

Structural analysis of the SRCD spectrum of **HZ wt** in 50% TFE.

3_{10}-helical structure in various membrane models, whereas deconvolution suggests a predominant α-helical conformation

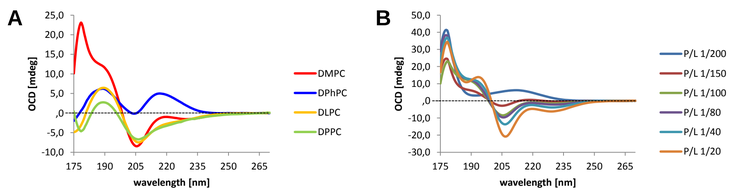
Peptide orientation by ssNMR and OCD



Solid-state ^{31}P- and ^{19}F-NMR spectra and observed ^{19}F-NMR dipolar splittings of ^{19}F-labeled **HZ** analogues in oriented DMPC and DPhPC bilayers; dotted line: isotropic position.

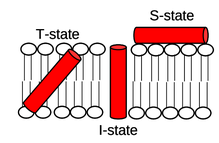
Structure model	Lipid	τ [°]	ρ [°]	S_{mol}	RMSD
3_{10}-helix (ideal)	DMPC	126	172	0.4	2.05
	DPhPC	78	10	0.5	2.0
α-helix (ideal)	DMPC	92	116	0.5	2.23
	DPhPC	96	102	0.5	**0.59**
β-bend ribbon spiral [7]	DMPC	102	118	0.6	1.38
	DPhPC	124	106	0.4	**0.87**

Putative alignment (τ and ρ angles) and dynamics (S_{mol}) of **HZ** in DMPC and DPhPC lipid bilayers assuming different models for secondary structure.



SR-OCD spectra of **HZ wt** in oriented phosphatidylcholine membranes of different composition (P/L 1/100) (**A**); **HZ wt** in oriented DMPC bilayers at varying P/L (**B**).

DMPC: β-bend ribbon and S-state
DPhPC: α-helix and S-state
OCD suggests two different states



Outlook

- Structure determination of **HZ wt** by NMR in solution
- Synthesis of ^{15}N-labeled **HZ** peptides to get more information on peptide alignment in lipid bilayers
- Analysis of membrane thinning (^{2}H-NMR, MD simulations)
- Channel conductance measurements

References:

[1] S. Rebuffat, S. Hlimi, Y. Prigent, C. Goulard, B. Bodo, J. Chem. Soc., Perkin Trans. 1 (1996) 2021; [2] N. Kučerka, M. Nieh, J. Katsaras, BBA Biomembr 1808 (2011) 2761-2771; [3] F. Huguenot, T. Brigaud, J. Org. Chem. 71 (2006) 7075–7078; [4] D. Maisch, P. Wadhwani, S. Afonin, C. Böttcher, B. Koksch, A.S. Ulrich, J. Am. Chem. Soc. 131 (2009) 15596–15597; [5] P.K. Mikhailiuk, S. Afonin, A.N. Chernega, E.B. Rusanov, M. Platonov, G. Dubinina, M. Berditsch, A.S. Ulrich, I.V. Komarov, Angewandte Chem. 118 (2006), 5787-5789; [6] A.S. Ulrich, Progress in Nuclear Magnetic Resonance Spectroscopy 46 (2005) 1–21; [7] I. Segalas, Y. Prigent, D. Davoust, B. Bodo, S. Rebuffat, Biopolymers (1999) 71–85.

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