Structure-function analysis of the peptaibol Harzianin HK-VI

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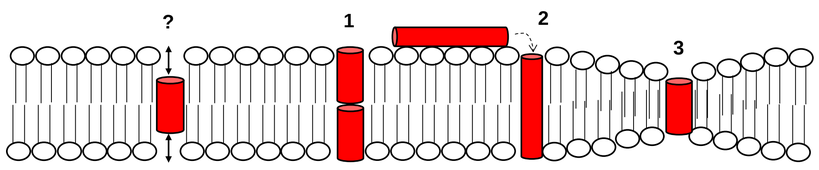
Introduction

Peptaibols are naturally occurring peptides isolated from fungal sources. They contain a high amount of **Aib (U)** (α-aminoisobutyric acid), possess a C-terminal alcohol and N-terminal acetylation/alkylation. They are able to permeabilize bacterial membranes by pore formation. **Harzianin HK-VI (HZ)** is an short peptaibol (11-mer) isolated from *Trichoderma pseudokoningii*.[1]

Aims

We aim to solve the structure of membrane-bound **HZ** and understand its interactions with lipid bilayers.



Hypothetical mechanisms how short pore-forming peptides can interact with lipid bilayers: by forming double-layered channels (**1**), by changing to an extended conformation (**2**), by causing membrane thinning (**3**).

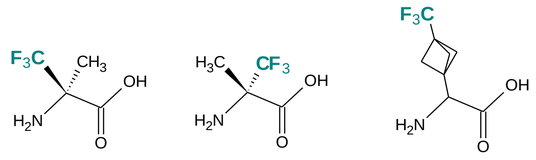
Methods

Solid-state ^{19}F-NMR and circular dichroism (CD) spectroscopy are used to determine the structure and alignment of **HZ** in lipid bilayers. For ^{19}F-NMR analysis, we use α-trifluoromethylated (Tfm) amino acids **(*R*)-, (*S*)-Tfm-Ala** [2,3] and **Tfm-Bpg** [4] as substitutes of **Aib** and **Leu/Ile** residues, respectively.

Sequence, labelling strategy and the labels:

N 1 2 3 4 5 6 7 8 9 10 11 C

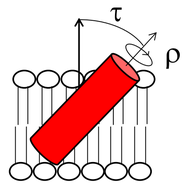
Ac- U N I I U P L L U P L -ol



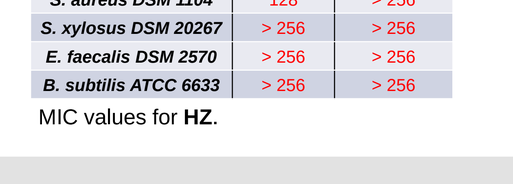
(*R*)-Tfm-Ala **(*S*)-Tfm-Ala** **Tfm-Bpg**

Peptide	Sequence
HZ	Ac-U-N-I-I-U-P-L-L-U-P-L-ol
HZ 1-(*R*)-Tfm-Ala	Ac-**(*R*)-Tfm-Ala**-N-I-I-U-P-L-L-U-P-L-ol
HZ 1-(*S*)-Tfm-Ala	Ac-**(*S*)-Tfm-Ala**-N-I-I-U-P-L-L-U-P-L-ol
HZ 5-(*R*)-Tfm-Ala	Ac-U-N-I-I-**(*R*)-Tfm-Ala**-P-L-L-U-P-L-ol
HZ 5-(*S*)-Tfm-Ala	Ac-U-N-I-I-**(*S*)-Tfm-Ala**-P-L-L-U-P-L-ol
HZ 9-(*R*)-Tfm-Ala	Ac-U-N-I-I-U-P-L-L-**(*R*)-Tfm-Ala**-P-L-ol
HZ 9-(*S*)-Tfm-Ala	Ac-U-N-I-I-U-P-L-L-**(*S*)-Tfm-Ala**-P-L-ol
HZ 3-Tfm-Bpg	Ac-U-N-**Tfm-Bpg**-I-U-P-L-L-U-P-L-ol
HZ 4-Tfm-Bpg	Ac-U-N-I-**Tfm-Bpg**-U-P-L-L-U-P-L-ol
HZ 7-Tfm-Bpg	Ac-U-N-I-I-U-P-**Tfm-Bpg**-L-U-P-L-ol
HZ 8-Tfm-Bpg	Ac-U-N-I-I-U-P-L-**Tfm-Bpg**-U-P-L-ol

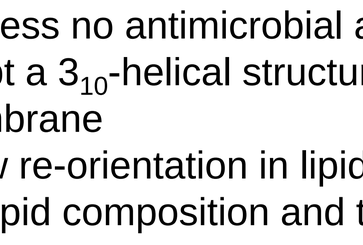
Synthetic peptides are reconstituted in oriented lipid bilayers. From the ^{19}F-NMR dipolar couplings, the structure and membrane alignment of peptides is determined.[5] Oriented CD with the synchrotron UV/VIS light source ANKA complements the results for the overall alignment of peptides.



Results



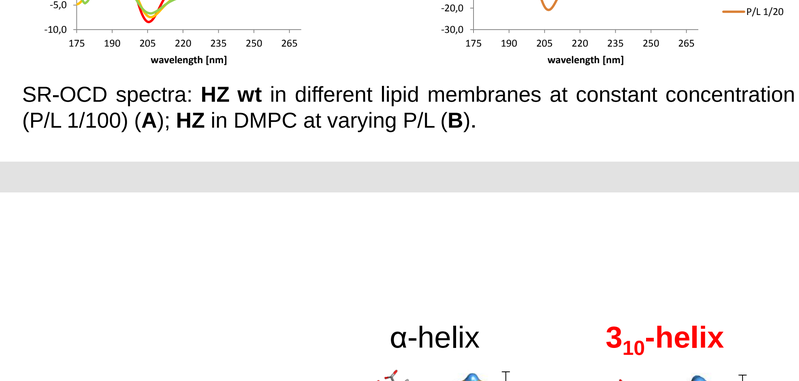
HPLC profiles of synthesized peptides: **HZ wt** crude (**A**) and pure (**B**), **HZ 1-(*S*)-Tfm-Ala** crude (**C**) and pure (**D**).



Representative MIC experiment. Strain: *B. subtilis ATCC 6633*. ALM: Alamethicin F30/3; GS: Gramicidin S; SC: solvent control; PC: positive control; NC: negative control.

Strain	MIC [µg/ml] HZ wt	MIC [µg/ml] HZ Tfm-Ala peptides
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

MIC values for **HZ**.

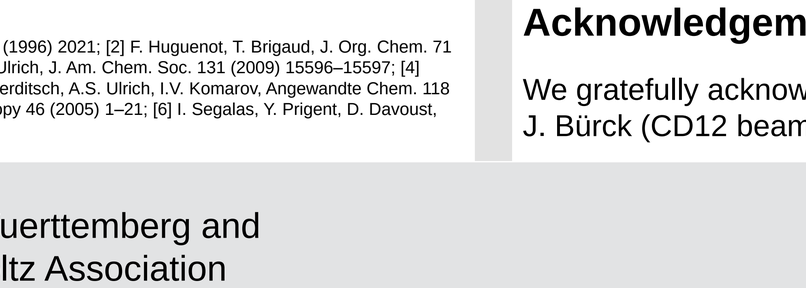


CD spectra of **HZ** peptides in H_2O (**A**) and TFE (**B**); **HZ wt** (**C**) and **HZ 5-(*S*)-Tfm-Ala** (**D**) in DMPC vesicles (P/L 1/20).

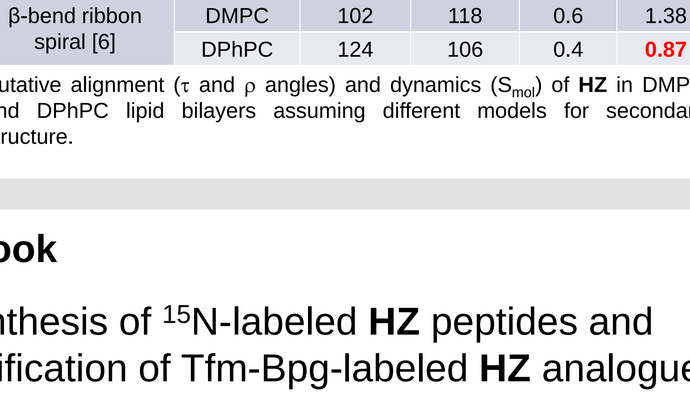
➢ 3_{10}-helical conformation

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

Deconvolution of the SR-CD spectrum for **HZ wt** in 50% TFE.



SR-OCD spectra: **HZ wt** in different lipid membranes at constant concentration (P/L 1/100) (**A**); **HZ** in DMPC at varying P/L (**B**).



^{31}P- and ^{19}F-NMR spectra of Tfm-Ala-labeled **HZ** analogues in oriented DMPC lipid bilayers (P/L 1/100) as a function of temperature.

Dipolar splitting [kHz]						
Lipid	1-(*R*)	1-(*S*)	5-(*R*)	5-(*S*)	9-(*R*)	9-(*S*)
DMPC	0	4.9	7.4	-2.7	0	-4.0
DPhPC	-1.2	5.2	4.8	0	4.6	-4.0

CF_3 dipolar splitting values of Tfm-Ala labeled **HZ** peptides in DMPC and DPhPC lipid bilayers (P/L 1/100).

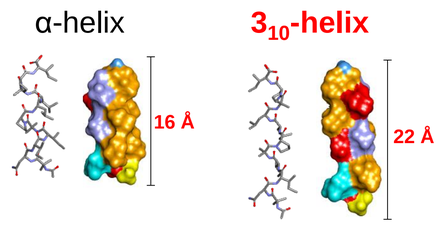
Structure model	Lipid	τ [°]	ρ [°]	S_{mol}	RMSD
3_{10}-helix (ideal)	DMPC	126	172	0.4	2.05
	DPhPC	78	10	0.5	2.0
α-helix (ideal)	DMPC	92	116	0.5	2.23
	DPhPC	96	102	0.5	**0.59**
β-bend ribbon spiral [6]	DMPC	102	118	0.6	1.38
	DPhPC	124	106	0.4	**0.87**

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

Conclusions

HZ peptides

- possess no antimicrobial activity
- adopt a 3_{10}-helical structure when bound to the lipid membrane
- show re-orientation in lipid membranes, dependent on the lipid composition and the lipid phase state



Outlook

- Synthesis of ^{15}N-labeled **HZ** peptides and purification of Tfm-Bpg-labeled **HZ** analogues to obtain more NMR constraints
- Investigation of the possibility of membrane thinning (^{2}H-NMR, MD simulations)
- Structure analysis of free **HZ** by NMR in solution and/or X-ray crystallography

References:

[1] S. Rebuffat, S. Hlimi, Y. Prigent, C. Goulard, B. Bodo, J. Chem. Soc., Perkin Trans. 1 (1996) 2021; [2] F. Huguenot, T. Brigaud, J. Org. Chem. 71 (2006) 7075–7078; [3] D. Maisch, P. Wadhwani, S. Afonin, C. Böttcher, B. Koksch, A.S. Ulrich, J. Am. Chem. Soc. 131 (2009) 15596–15597; [4] 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; [5] A.S. Ulrich, Progress in Nuclear Magnetic Resonance Spectroscopy 46 (2005) 1–21; [6] I. Segalas, Y. Prigent, D. Davoust, B. Bodo, S. Rebuffat, Biopolymers (1999) 71–85.

Acknowledgements

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