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Deciphering the Role of Citrulline and Cyclization in the Rational Design of Antimicrobial Peptides Derived from Equinin B

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Introduction: Equinin B is an antimicrobial peptide (AMP) isolated from the cnidarian *Actinia equina* (Linnaeus, 1758), an anthozoan coelenterate of the family Actiniidae distributed from the Indo-Pacific to the Mediterranean and the Atlantic. The amino acid sequence of equinin B, GQCQRKCLGHCSKKCPKHPQCRKRCIRRCFGYCL, consists of 34 amino acids and shows 37.78% similarity to another AMP, aurelin, isolated from jellyfish.[1] We prepared truncated peptides from equinin B to identify the smallest fragment that still retains antimicrobial activity.[2] Subsequently, we prepared derivatives of these three fragments. First, we substituted Lys with Arg (**EB1-K**, **EB2-K**), then we substituted Cys with Arg (**EB1-C**, **EB2-C**), and after that all Cys were substituted with Cit (**EB1-Cit, EB2-Cit, EB3-Cit**). Finally, cyclization was achieved with disulfide bridges[3] (**EB1-Cyc, EB2-Cyc, EB3-Cyc**) and by inserting bipyridine-dicarboxylic acid (**EB3-Py**) (Table 1).

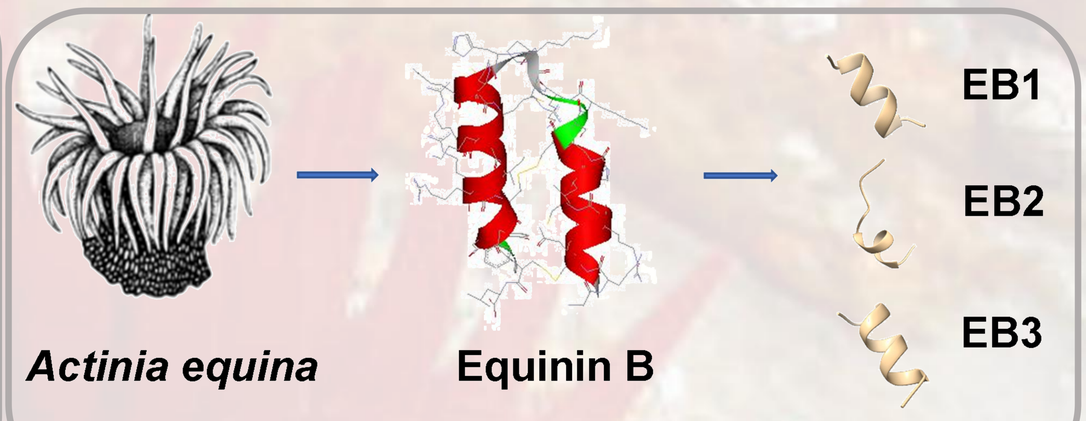


Table 1. Basic characteristics of the peptides used in the study

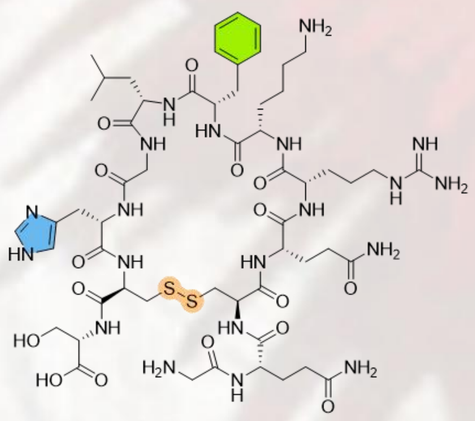
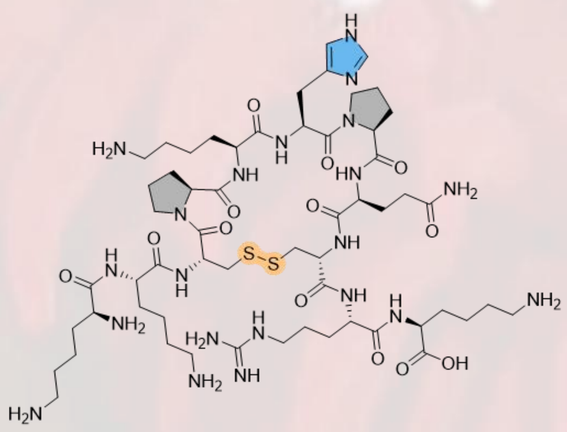
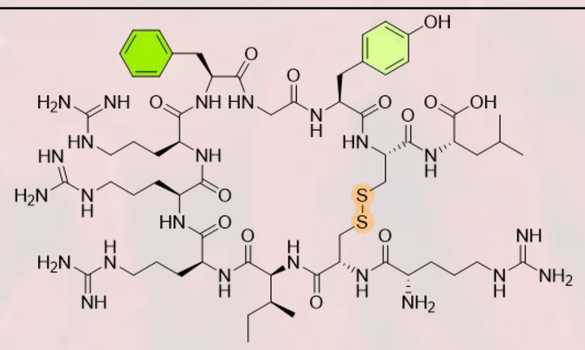
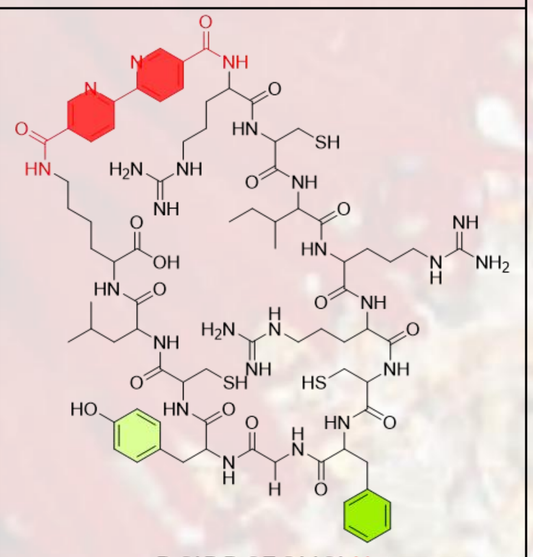
GQCQRKCLGHCSKKCPKHPQCRKRCIRRCFGYCL **Equinin B**		
Fragment 1	**Fragment 2**	**Fragment 3**
GQCQRKCLGHCS **EB1**	KKCPKHPQCRK **EB2**	RCIRRCFGYCL **EB3** ANTIMICROBIAL, CYTOTOXIC
Equinin B derivatives		
GQCQRRCLGHCS **EB1-K** ANTIFUNGAL (BF)	RRCPRHPQCRR **EB2-K** ANTIFUNGAL (BF)	
GQRQRKRLGHRS **EB1-C**	KKRPKHPQRRK **EB2-C** ANTIFUNGAL (BF)	RRIRRRFGYRL **EB3-C** ANTIBACTERIAL, NON-CYTOTOXIC
GQCitQRKCitLGHCitS **EB1-Cit**	KKCitPKHPQCitRK **EB2-Cit**	RCitIRRCitFGYCitL **EB3-Cit** INACTIVE
 GQCQRKCLGHCS **EB1-Cyc**	 KKCPKHPQCRK **EB2-Cyc**	 RCIRRCFGYCL **EB3-Cyc** INSOLUBLE
		 RCIRRCFGYCLK **EB3-Cyc-Py** INSOLUBLE

Table 2. Antimicrobial activity

Comp.	*E.coli*		*S.epidermidis*		*S. aureus*		*E. faecalis*		*P.aeruginosa*		*C. albicans*	
	MIC	IBF	MIC	IBF	MIC	IBF	MIC	IBF	MIC	IBF	MIC	IBF
EB1	>400	>400	>100		>400	>400	>400	>400	>400	>400	>400	>400
EB2	>400	>400	>100		>400	>400	>400	>400	>400	>400	>400	>400
EB3	200	>400	25		>400	200	>400	200	>400	>400	200	150
EB1-K	>400	>400	>100		>400	200	>400	>400	>400	>400	>400	200
EB2-K	>400	>400	>100		>400	>400	>400	>400	>400	>400	>400	100
EB1-C	>100		>100									
EB2-C	>100		>100									
EB3-C	>100		25-50									
EB1-Cit	>1200		>1200									
EB2-Cit	>1200		>1200									
EB3-Cit	>1200		>1200									
EB1-Cyc	>1200		>1200									
EB2-Cyc	>1200		>1200									

MIC = minimum inhibitory concentration (μg/mL)
IBF = inhibition of biofilm formation (μg/mL)

Conclusion:

- Truncated peptides of equinin B, a 34 amino acid peptide, were synthesized by Fmoc solid phase peptide synthesis (SPPS)
- Cyclization was acheved either via a disulfide bridge or with bipyridine-dicarboxylic acid
- The main AM activity was observed in the third part of the peptide, EB3
- Citrullinated EB3-Cit lost the activity observed in EB3 and EB3-C
- Cyclic derivatives obtained either with a disulfide bridge or with a bipyridine bridge were not soluble and could not be used
- Interesting results were obtained with compounds EB1-K and EB2-K, which exhibited activity against biofilm formation (Table 2)
- Further investigation of compounds for biofilm activity should be carried out with other synthesized compounds, especially the cyclic ones

References:

[1]C. La Corte et al., *Mar. Drugs* 2024, 22, 172. https://doi.org/10.3390/md22040172
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[3]T. M. Postma and F. Albericio, *Eur. J. Org. Chem*. 2014, 3519–3530. https://doi.org/10.1002/ejoc.201402149

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