

Microwave Solid Phase Synthesis of Natriuretic Peptide Sequences Derived from the Venom of Native and Exotic Lizards

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Introduction

Prior to 2006 it was thought that advanced snakes and helodermatid lizards (Gila monster and Bearded lizard) were the only two reptile lineages that possessed venom delivery systems, and the lizard system was thought to have evolved independently from the snake venom system [1]. Recent phylogenetic analysis of sequence data from cDNA libraries constructed from the mandibular and maxillary glands of anguimorph and iguanian lizards (which includes the heladermatids) has shown that they are the closest relatives of snakes. Together they represent a well-resolved venomous clade possessing protein-secreting oral glands. Sequencing identified nine different toxin sequences present in each venomous lizard species.

ANP/BNP-like peptide [2] is one of the toxin-types identified, and the sequences derived from seven different anguimorph lizards have been synthesized on a CEM Liberty Microwave peptide synthesiser and tested for activity in the aortic ring relaxation assay [3]. The natriuretic peptide sequence from Ophisaurus apodus (Oplo), when compared to BNP, has the substitutions Glu(E) for Asp(D)

and Val(V) for Ile(I). The Oplo-D, Oplo-I and Oplo-DI analogues were synthesized as part of the natriuretic peptide set.

Results and Discussion

The peptide sequences were between 32-38 amino acids in length with a single disulfide bond. Synthesis was performed on a CEM Liberty microwave peptide synthesiser on a 0.1 mmole scale. Yield and purity of the crude linear peptides depended on the resin used. All peptides were synthesized as amides on a Rink derivatized resin. The best results were obtained on Fmoc-Pal-PEG-Resin. Crude peptides were RP HPLC purified to >90% in the linear form. The purified linear peptides were cyclized to the disulfide bonded molecule by treatment with dipyridyldithiol (Aldrithiol, 1 equivalent, 30 minutes) in ammonium acetate buffer, pH6.5.

The cyclised peptides were HPLC purified under reversed phase conditions complementary to those employed for the initial linear purification. The purity of the final products was >95% pure peptide yields were in the range 1-12% depending on the synthetic conditions (resin).

Alignment of ANP and BNP sequences

ANP	human		S	L	R	R	S	S	C	F	G	G	R	M	D	R	I	G	A	Q	S	G	L	G	C	N	S	F	R	Y										
ANP	rat		S	L	R	R	S	S	C	F	G	G	R	I	D	R	I	G	A	Q	S	G	L	G	C	N	S	F	R	Y										
BNP	human		S	P	K	M	V	Q	G	S	G	C	F	G	R	K	M	D	R	I	S	S	S	S	G	L	G	C	K	V	L	R	R	H						
BNP	rat	I	Q	E	R	L	R	N	S	K	M	A	H	S	S	S	C	F	G	Q	K	I	D	R	I	G	A	V	S	R	L	G	C	D	G	L	R	L	F	
OPLO-EV			Y	P	G	D	G	C	F	G	Q	K	I	E	R	V	G	T	V	S	G	M	G	C	R	S	S	N	T	G	Y	S	G	K	K					
OPLO-D			Y	P	G	D	G	C	F	G	Q	K	I	D	R	V	G	T	V	S	G	M	G	C	R	S	S	N	T	G	Y	S	G	K	K					
OPLO-I			Y	P	G	D	G	C	F	G	Q	K	I	E	R	I	G	T	V	S	G	M	G	C	R	S	S	N	T	G	Y	S	G	K	K					
OPLO-DI			Y	P	G	D	G	C	F	G	Q	K	I	D	R	I	G	T	V	S	G	M	G	C	R	S	S	N	T	G	Y	S	G	K	K					
VKOM			I	Q	P	E	G	S	C	F	G	Q	K	I	D	R	I	G	H	V	S	G	M	G	C	N	K	F	D	P	N	K	E	S	S	S	T	G		
HSUS-NP			L	S	P	G	D	G	C	F	G	Q	K	I	D	R	I	G	A	V	S	G	M	G	C	N	S	V	S	S	Q	G	K	K						
CWAR			H	Y	P	G	N	G	C	F	G	Q	K	I	D	R	I	G	A	V	S	G	M	G	C	R	G	S	I	M	R	P	Q	P	G	R	D	A		
VGLA_CL5	CONTIG1		L	Q	P	G	E	S	C	F	G	Q	K	I	D	R	I	G	H	V	S	G	M	G	C	N	T	F	D	P	N	K	G	S	S	S	T	E	K	K
VSCA_CL5	CONTIG1		L	Q	P	G	D	S	C	F	G	Q	K	I	D	R	I	G	H	A	S	G	M	G	C	N	T	F	D	P	N	K	E	S	S	S	A	E	K	K
VGLA_CL2	CONTIG1		L	S	P	G	S	G	C	F	G	N	R	L	D	R	I	G	S	T	S	G	L	G	C	G	S	G	S	S	S	G	T	G	T	A	G	K	K	

Table 1. Alignment of ANP, BNP and Lizard natriuretic peptide sequences

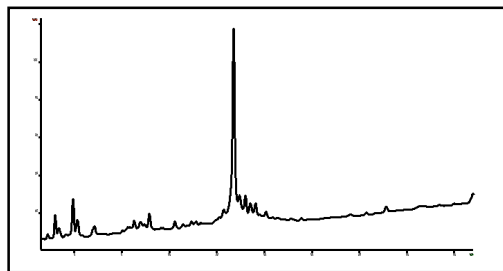
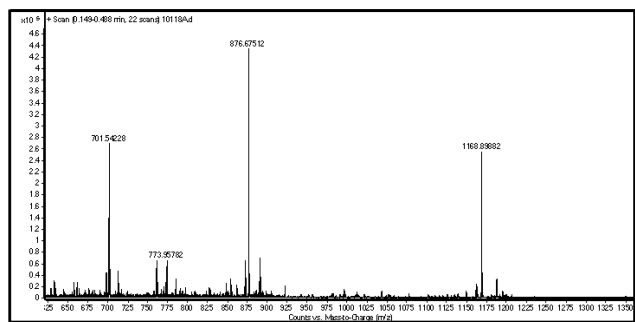


Fig. 1. Example of crude linear natriuretic peptide HPLC trace and mass spectrum from *Varanus glauerti*, the Kimberly rock monitor

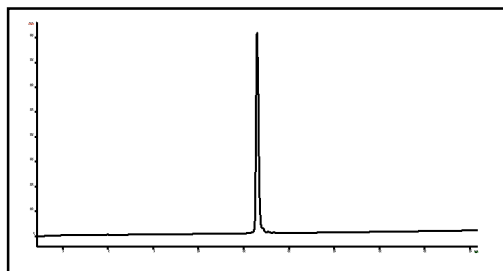
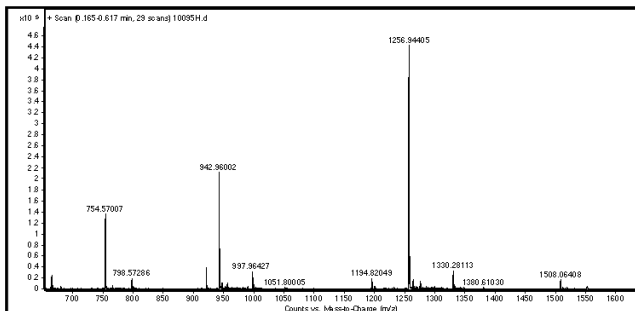


Fig. 2. Example of pure cyclic natriuretic peptide HPLC trace and mass spectrum from *Celestes warreni*, the Haitian Galliwasp

All peptides were tested for activity in a rat aortic ring relaxation assay. Briefly, aortic rings from male Sprague-Daly rats were mounted in organ baths and the tissues were pre-contracted with phenylephrine. Ring preparations were conditioned through two cycles of contraction/relaxation with 40mM KCl and then tested with different doses of natriuretic peptide. Responses were calculated as a percentage relaxation of the KCl response.

All the anguimorph natriuretic peptides displayed activity in this assay. Asp/Ile substitutions in the Oplo sequence were all more active than the native sequence.

E - tissues pre contracted with phenylephrine

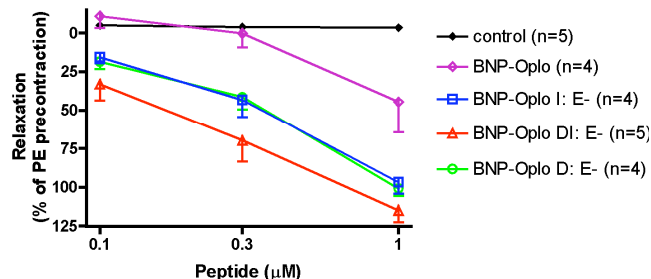


Fig.3. Comparison of the relative relaxation ability of single amino acid substitutions in the natriuretic peptide sequence obtained from *Ophisaurus opodus*, the European legless lizard

In summary, these results open the way for the discovery of new bioactive and potentially therapeutic peptide molecules from a rich and currently unexplored source, namely anguimorph lizard venom.

Acknowledgments

We thank Bio21 Institute and The University of Melbourne for their financial support.

References

1. Fry BG, Vidal N, Norman JA, Vonk FJ, Scheib H, Ramjan R, Kuruppu S, Fung K, Hedges SB, Richardson MK, Hodgson WC, Ignjatovic V, Summerhayes R and Kochva E (2006) *Nature* 439 (7076): 584-588; Advance online publication November 17, 2005 doi:10.1038/nature04328
2. Atlas, S.A., and Laragh, J.H. (1986) *Ann. Rev. Med.* 37: 379-414.
3. Fry, B.G., Wickramaratana, J.C., Lemme, S., Beuve, A., Garbers, D., Hodgson, W.C., and Alewood, P. (2005) *BBRC* 327, 1011-1015.