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Mechanistic Proposal for Restricted Peptides Action on Parasite Membrane

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Introduction

Malaria is an infectious disease responsible for approximately one million deaths annually. Peptides such as angiotensin II (AII) and its analogs are known to have antimalarial effects against *Plasmodium gallinaceum* [1] and *Plasmodium falciparum* [2]. However, their mechanism of action is still not fully understood at the molecular level. In this work, we investigated this issue by comparing the antimalarial activity of angiotensin II with that of: *i*) its enantiomer formed by only D-amino acids; *ii*) its isomer with reversed sequence; and *iii*) its analogs restricted by lactam bridges - the so-called VC5 peptides.

Results and Discussion

The peptides were synthesized manually with the *t*-Boc strategy on Merrifield resin (Table 1). AII could inactivate 88% of the *Plasmodium gallinaceum* sporozoites, which is consistent with its known antimalarial properties. Similar anti-plasmodial effects were measured for ent-AII and other peptides (Figure 1). In contrast to AII, *ent*-AII and *retro*-AII could not interact with the membrane AII-receptors (Figure 2).

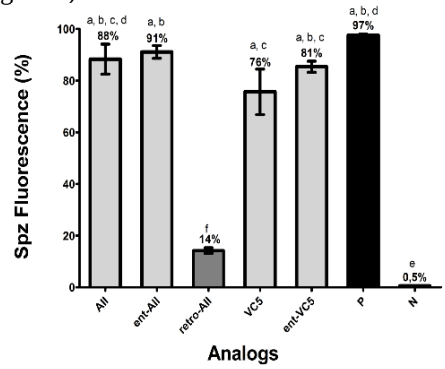

Fig. 1. Effects of AII analogs on membrane permeability expressed as the percent of fluorescent mature sporozoites (mean ± standard deviation of 9 independent measurements). Letters indicate the results that are not significantly different from each other at the $p < 0.05$ level. Positive control group (+): digitonin/PBS; negative control group (–): PBS.

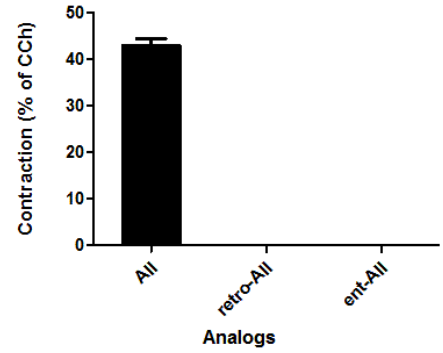

Fig. 2. Effects of peptides on contractile responses during muscle tissue incubation compared to carbachol (CCh) activity. AT_1 receptor recognition was analyzed via pre-incubation with Losartan (mean ± standard deviation, N = 2).

This indicates that the anti-plasmodial effects of AII analogs depend on direct peptide-phospholipid interactions. This hypothesis was also supported by the anti-plasmodial activities recorded for the lactam bridge-restricted analogs VC5 and *ent*-VC5. These were comparable to that of AII. A significant change in the anti-plasmodial activity was observed only for *retro*-AII, which was ~6-fold less effective than AII. We used CD experiments to show that the β-turn was the most frequent

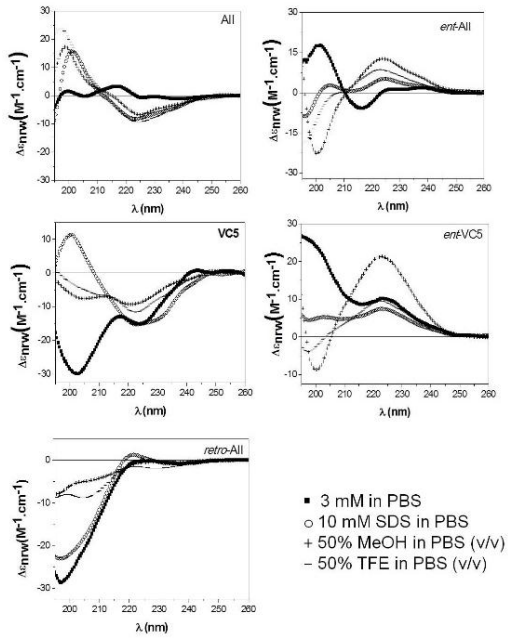

Fig. 3. Circular dichroism spectra of AII analogs. All peptides were analyzed in the following four solutions: 15 mmol L^{-1} PBS, 10 mmol L^{-1} SDS/PBS, 50% TFE/PBS, and 50% MeOH/PBS. The peptide concentration was approximately 10^{-4} mol L^{-1}.

conformation adopted by peptides in aqueous and organic solvents. Moreover, the β-turn conformation was correlated with a larger anti-plasmodial activity.

In the presence of SDS micelles, AII had a β-turn conformation while *retro*-AII presented a random coiled conformation (Figure 3). Consistently, molecular dynamic simulations revealed that the AII chains were slightly more bent than *retro*-AII at the surface of a model phospholipid bilayer (Figure 4). We did not observe spontaneous pore formation in either case. This may be an indication that this process involves larger time scales and possibly the organization of the peptide chains into larger assemblies. However, qualitative differences were identified between the behavior of AII and *retro*-AII by pulling both peptides across the phospholipid bilayer. At the hydrophobic membrane interior, the *retro*-AII chain was severely coiled and rigid. AII was much more flexible and could experience both straight and coiled conformations. Interactions between AII and phospholipid head groups were kept for a longer time even in the membrane interior. It is conceivable that stronger peptide-head group interactions might be more effective at stabilizing a pore in longer time scales. This contributes to the larger anti-plasmodial activity of AII versus *retro*-AII. We hope that our results can be used for the systematic design of novel compounds with antimalarial activity.

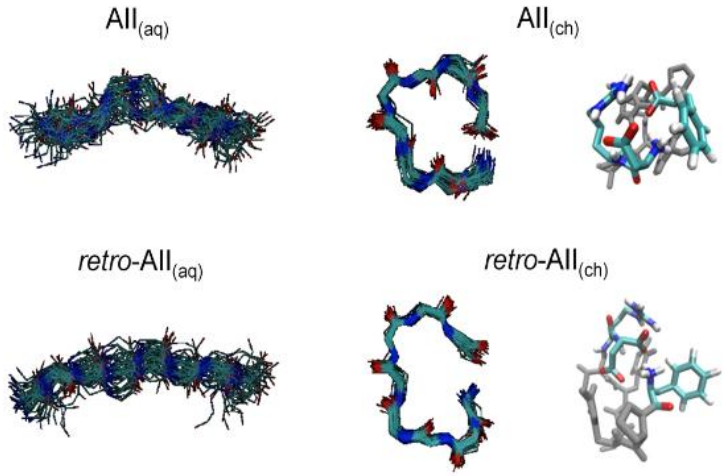

Fig. 4. Conformations of the backbone atoms of AII and retro-AII in water (left) and cyclohexane (center). The conformations recorded during the last 5 ns of simulation were overlaid. Selected conformers in cyclohexane (right) are represented in grey with Asp, Arg and Phe residues highlighted in color. Color code: C (cyan), N (blue), O (red), H (white).

Table 1. Mass characterization and purities of peptides synthesized.

Peptide	*Sequence*	*HPLC Purity (%)*[a]	*Mass Characterization*	
			Calculated Mass (Da)	*Observed Mass (m/z)*[b]
AII	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe	-	1045.5	-
ent-AII	DAsp-DArg-DVal-DTyr-DIle-DHis-DPro-DPhe	99	1045.5	1047.5
retro-AII	Phe-Pro-His-Ile-Tyr-Val-Arg-Asp	100	1045.5	1047.5
VC5	Asp-Arg-Asp-Val-Lys-Tyr-Ile-His-Pro-Phe	95	1271.5	1272.5
ent-VC5	DAsp-DArg-DAsp-DVal-DLys-DTyr-DIle-DHis-DPro-DPhe	99	1271.5	1272.5

[a]*HPLC: Column Supelcosil C18 (4.6 x 150 mm), 60 Å, 5 μm; Solvent System: A (0.1% TFA/H2O) and B (0.1% TFA in 60% ACN/H2O); Gradient: 5-95% B in 30 minutes, Flow: 1.0 mL/min; λ = 220 nm; Injection Volume: 50 μL and Sample Concentration: 1.0 mg/mL.*

[b]*LC/ESI-MS: Micromass instrument, model ZMD coupled on a Waters Alliance, model 2690 system. Conditions of mass measurements: positive mode; range between 500 and 2000 m/z; nitrogen gas flow: 4.1 L/h; capillary: 2.3 kV; cone voltage: 32 V; extractor: 8 V; source heater: 100 ºC; solvent heater: 400ºC; ion energy: 1.0 V and multiplier: 800 V.*

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