New Drug Development Group

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Development of Effective Antagonists against ELR-CXC Chemokine Receptors to Inhibit Prostate Cancer and Acute Lung Injury Caused by Influenza Virus Infection: Structural and Functional Studies of $CXCL8_{(3-72)}$K11R/G31P

Abstract

Prostate cancer is one of the most malignancy in Western World. The ELR-CXC chemokines are important to neutrophil inflammation in many acute and chronic diseases. Among them, CXCL8 (interleukin-8, IL-8), binds to both the CXCR1 and CXCR2 receptors with high affinity and the expression levels of CXCL8 are elevated in many inflammatory diseases. Furthermore, CXCR1/2 also play crucial roles in the prostate cancer development and progression. Recently, an analogue of human CXCL8, $CXCL8_{(3-72)}$K11R/G31P (hG31P) has been developed. It has been demonstrated that hG31P is a high affinity antagonist for both CXCR1 and CXCR2. To obtain large quantities of hG31P, we have successfully constructed and expressed hG31P in *Escherichia coli*. Moreover, we have developed a new protocol for high-yield purification of hG31P and for the removal of lipopolysaccharide (LPS, endotoxin) associated with hG31P due to the expression in *Escherichia coli*. The purity of hG31P is more than 95% and the final yield is 9.7 mg hG31P per gram of cell paste. The purified hG31P was tested by various biological assays. Additionally, G31P treatment of nude mice suppressed the growth of orthotopically transplanted tumor xenografts. The structural properties of hG31P were studied by circular dichroism (CD), ultracentrifuge, isothermal titration calorimetry (ITC), and nuclear magnetic resonance (NMR) spectroscopy. We have also identified the binding sites of human $CXCL8_{(3-73)}$K11R/G31P to a CXC chemokine receptor fragment by chemical shift perturbation. Our results provide a structural basis for future clinical investigations of this CXCR1/CXCR2 receptor antagonist.

Key words: CXCL8, Antagonist, Expression, Purification, Lipopolysaccharide

Results

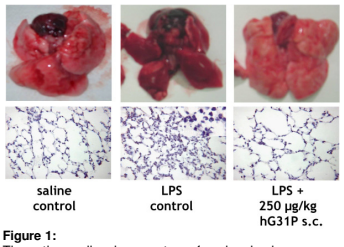
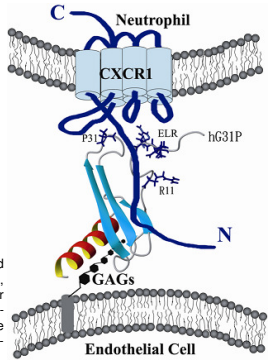


Figure 1:
The entire cardiopulmonary tree of each animal was removed and photographed using a ventral approach (upper panel), then subjected to BAL, and processed to paraffin sections for histopathology (lower panel). The lungs of the LPS-challenged animals which were treated with hG31P were markedly less hemorrhagic and inflamed than the saline-treated animals.



Strategy to Increase Salt Resistance of Antimicrobial Peptide

Abstract

Antimicrobial peptides play important roles in the host innate defense mechanism by interacting and permeabilizing microbial membranes. With an increase of antibiotic resistance, the potential for the development of antimicrobial peptides as novel therapeutic agents could overcome the problem of resistance. However, the efficacies of many antimicrobial peptides are greatly reduced under high salt concentrations, limiting their development as pharmaceutical compounds. Here, we describe an easy strategy to increase salt resistance of antimicrobial peptides by replacing tryptophan or histidine residues with the bulky amino acids beta–naphthylalanine and beta-(4,4-biphenyl)alanine. The activities of the salt-sensitive peptide P-113 were diminished at high salt concentrations, whereas the activities of its -naphthylalanine and beta-(4,4-biphenyl)alanine-substituted variant were less affected.

Moreover, we develop a series of strategy to investigate the relationship between orientation and salt resistance of tryptophan-rich antimicrobial peptides by NMR methods. PEM-2-W5K/A9W, a short Trp-rich antimicrobial peptide developed based on the structural studies of PEM-2, has been shown to be highly active against various bacterial strains with less hemolytic activity. The antimicrobial activities of PEM-2-W5K/A9W against various bacterial and fungal strains including multidrug-resistant and clinical isolates under high salt conditions were tested. The activities of the salt-sensitive peptide P-113 and PEM-2 were reduced and diminished at high salt concentrations, whereas the activities of PEM-2-W5K/A9W were less affected.

Key words: antimicrobial peptide, salt resistance, tryptophan-rich, histidine, bulky amino acid, paramagnetic relaxation enhancement, saturation transfer difference.

Table 1. MIC (μg/ml) values for peptides in different concentrations of NaCl.

Species	MH			LYM														
				control			NaCl											
							50 mM			100 mM			200 mM			300 mM		
	P-113	PEM-2	W5K/A9W	P-113	PEM-2	W5K/A9W	P-113	PEM-2	W5K/A9W	P-113	PEM-2	W5K/A9W	P-113	PEM-2	W5K/A9W	P-113	PEM-2	W5K/A9W
E. coli ATCC 25922	>50*	6.25	0.78	12.5	3.1	0.78	50	6.25	1.56	50	25	1.56	>50	25	6.25	>50	50	12.5
S. aureus ATCC 25923	>50	25	6.25	12.5	1.56	0.78	50	3.1	1.56	50	12.5	12.5	>50	50	3.1	>50	>50	12.5
S. aureus ATCC 19636	>50	6.25	1.56	50	3.1	1.56	50	6.25	3.1	50	25	6.25	>50	25	3.1	>50	50	12.5
S. aureus ATCC 29213	>50	50	6.25	12.5	3.1	1.56	50	3.1	1.56	50	3.1	1.56	>50	12.5	3.1	>50	50	6.5
P. arugenosa ATCC 27853	>50	12.5	3.1	25	3.1	1.56	50	25	1.56	50	50	1.56	>50	>50	3.1	>50	>50	12.5
P. arugenosa ATCC 9027	>50	12.5	0.78	12.5	1.56	0.78	50	12.5	3.1	50	50	3.1	>50	>50	6.25	>50	>50	12.5

*MIC (μg mL^{-1})

MIC, Minimal Inhibition Concentration.

Results

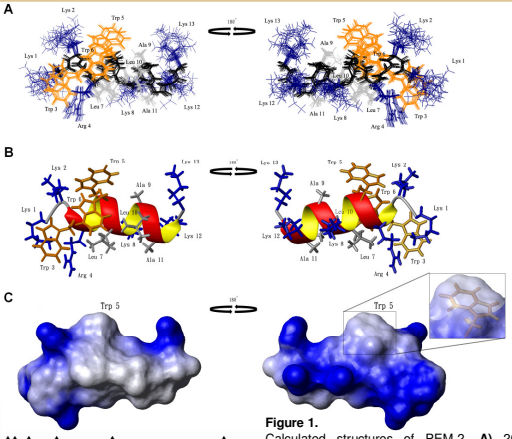


Figure 1.
Calculated structures of PEM-2. **A)** 20 lowest energy structures calculated for PEM-2. **B)** The final refined average structure for PEM-2. The positive charged residues are labeled in blue, the tryptophan residues are labeled in brown, and the hydrophobic residues are labeled in gray. **C)** The electrostatic surface plot of PEM-2. Positive charge is colored in blue and neutral charge is colored in white.

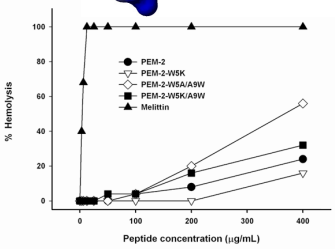


Figure 2.
Hemolytic activities of PEM-2 (●), PEM-2-W5K (▽), PEM-2-W5A/A9W (◇) and PEM-2-W5K/A9W (■). Melittin (▲) was used as a control.

In vitro selection and structural characterization of DNA aptamers of the domain III of dengue virus envelope protein

Abstract

The flavivirus envelope protein is the dominant antigen in eliciting neutralizing antibodies and plays an important role in inducing immunologic responses in the infected host. Among the three domains of the envelope protein, domain III plays the most important role in viral attachment and fusion. We have conducted a SELEX experiment and generated a DNA aptamer that targets domain III of the Dengue-2 virus envelop protein. Binding of this aptamer to DENV-2 domain III is determined by fluorescence spectroscopy and ELISA. We have also identified the binding sites of the aptamer on DENV domain III. Site-directed mutagenesis experiments are performed to confirm the structural results. Our results provide a new aptamer as an artificial antibody that targets the DENV envelop protein.

Key words: aptamer, SELEX, neutralize, envelope protein, Dengue virus.

Table 1. Sequence alignment of the random region of selected aptamers.

Aptamer	Sequence	Frequency
Group A (44%)*		
S22	CCGCACCACATCAGCCCGTCGTCGCCCTCGTCCG	19
S85	CCGCACCACATCAGCCCGTCGTCGCCCTCTTCCG	1
S91	CCGCACCACATCAGCCCGTTGTCGCCCTCCGTCG	1
S96	CCGCACCACATCGCCCCGCCGTCGCCCTCGACCA	1
Group B (20%)*		
S15	GCACCGAGCAGGACGTCCGGGGTCCTCGGGGGGC	12
S105	GCACCGAGCAGGACGTCCGGGGTCCTCGGGGGGC	1
S119	GCACCGGGCAGGGACGTCCGGGGGTCCTCGGGGGGT	1
Group C (14%)		
S55	GCCAGGAGCGTAGCGGCGCAGGAGGCGTGGCTGGT	7
Group D (8%)		
S23	CCCATCTAACAATTATCATCCTCCCCCTCCTCCCC	4
Group E (2%)		
S131	CGCCTCGCACCAGTTCATTATACCCCCCCTGTATG	1
Group F (2%)		
S161	GCACCACACTCGCATTGGATTCGGCCCCCATTTGG	1
Group G (2%)		
S165	CCAGGAGCGTAGCGGCGCAGGAGGCGTGGCTGGT	1

Results

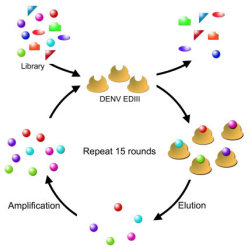


Figure 1.
Flowchart of SELEX procedure *in vitro*.

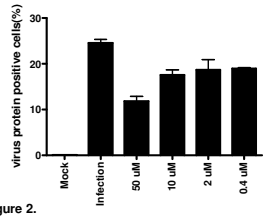


Figure 2.
Inhibition of DENV infection by S15 *in vivo*. Virus was incubated with S15 for 1 hour, and then was introduced to A549 cells. S15 was able to decrease 50% infection.

Reference

1. Yu HY, Yip BS, Tu CH, Chen HL, Chu HL, Chih YH, Cheng HT, Sue SC, **Cheng JW.** **Biochim Biophys Acta. (BBA) - Biomembranes**, Volume 1828, Issue 11, November 2013, Pages 2720-2728.
2. H.L. Chu, H.Y. Yu, B.S. Yip, Y.H. Chih, C.W. Liang, H.T. Cheng, **Cheng JW**. **Antimicrobial Agents and Chemotherapy.** 2013 Aug;57(8):4050-2.
3. Hui-Yuan Yu, Chih-Hsiang Tu, Bak-Sau Yip, Heng-Li Chen, His-Tsung Cheng, Kuo-Chun Huang, Hsiu-Jung Lo, and **Cheng JW**, ***Antimicrobial Agents and Chemotherapy***, **2011**, 55, 4918-4921.
4. Liu X, Peng J, Sun W, Yang S, Deng G, Li F, **Cheng JW**, Gordon JR. **Tohoku J Exp Med. 2012**;228(2):147-56.
5. H.T. Cheng, K.C. Hung, H.Y. Yu, K.J. Gao, X. Zhao, F. Li, J. Town, J.R. Gordon, **Cheng JW**, ***Prot. Express. Purif.*** **2008**, 61, 65-72.