The Design and Synthesis of Efficiency Peptides of Binding the Neurotoxins and Extension of Peptide Libraries

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Abstract: α-neurotoxin is a toxin isolated from most of snake poison. Scientists have developed methods to neutralize the toxin and to treat the poisoning patients. Studies have found a series of peptide which make it easier to combine with α-neurotoxin. Thus, in this way, α-neurotoxin would not be available to combine with nACHR where it causes the disease. This review discusses how the researchers have design and synthesis the peptide through focusing on the sample that have a high affinity of α-neurotoxins and replacing amino acids to receive a better affinity. Moreover, the library researchers have built to further study of peptides in treatment or other potential purposes are also discussed.

1. Introduction

Neuromuscular paralysis is one of the common effects after snake envenoming. It is often fatal when the effect spread to bulbar and respiratory muscles [1]. After searching for multiple venomous snakes and the genes that control them, researchers found that a lot of neuromuscular paralysis cases were found in snakes with high levels of α-neurotoxin [2]. The toxic mechanism of these snake venoms is the combination of α-neurotoxin and nicotinic acetylcholine receptor (nAChR) to form a complex, causing neuromuscular block [1].

α-neurotoxins are three-finger toxins of which the structure is derived from a core group to take the shape of three fingers. This structure has a strong correlation with its combination with nAChR to form a stable complex. However, the structure of nAChR has not been fully understood because of its hydrophobic property and large molecular weight, which makes it difficult to be detected by X-ray diffraction or mass spectrometry. Researchers have categorized α-neurotoxins into two groups, which are called long α-neurotoxins and short α-neurotoxins. The reversibility of long α-neurotoxins is weaker than that of short α-neurotoxins, as a result, leading to more problems [2].

This review discusses the studies that used methods of building lead peptide libraries to replace some amino acids to increase their affinity and construct high affinity peptides, of which structure is similar to the nAChR. More libraries database with the biological activities may be one of the critical solutions to further understand and to promote the peptide synthesis and potential clinical usage. A typical library about activity from chemical modification group of the peptides is discussed in the review.

2. Binding of three-finger toxin and HAPs

α-neurotoxin is a three-finger toxin which can be divided into 4 groups: short chain α-3FNTxs, long chain α-3FNTxs, non-conventional α-3FNTxs and dimeric α-3FNTxs. Even in the same α-3FNTxs group, the diversity of nAChR subtype selectivity, binding ability, and reversibility are different [1].

To a large extent, all α-3FNTxs inhibit muscular nAChR with different affinity, while LC-α-3FNTXs and NC-α-3fntxs also inhibit some neuronal nAChR subtypes. The dimer α-3FNTXS appears to show a wider range of nAChR selectivity than its constituent monomer α-3Fntx groups, suggesting that the dimer is a strategy for achieving a wider range of target selection. NC-a-FNTxs is a weak toxin, with LD_{50} of 5~80 mg/kg in animal bodies. Neurotoxicity of snake-bite injury can be attributed to the competitive inhibition of acetylcholine binding to postsynaptic muscle nAChRs by α-3FNTXs or other α-neurotoxins [3].

The presynaptic effects of PLA2-related toxins would cause a depletion of ACh synaptic vesicles and phospholipid hydrolysis, leading to irreversible destruction of motor nerve endings. Other mechanisms of PLA2 toxins action have also been proposed in binding voltage-gated potassium channels and interacting with muscle nAChRs. Some neurotoxins, such as dendritic toxins and bundle membrane proteins in mamba venom, could inhibit presynaptic voltage-gated potassium channels and synaptic acetylcholinesterase, respectively. As a result, they could lead to excitatory effects and beams [3].

HAPs is high affinity peptide which can combine with αBgt with a high affinity. In this property, HAP can be a remedy as an antidote to α-neurotoxins. αBgt, or α-bungarotoxin, is a venom from *bungarus multicinctus*. αCtx is α-Cobratoxin, which is a venom from cobra [3].

The first target of the experiment is to verify the theory how nAChR combine with the α-neurotoxins. Researchers used HAP which effected on 125I-αBgt as an example, and found that little doze of the HAP can combine with the venom with specific binding. Thus, existence of HAP showed a strong will to suppress 125I-αBgt binding to receptor in a dose-dependent manner [3].

Researchers have built the three-dimensioned model of αCtx–HAP complex and αBgt–HAP complex and discovered the difference between these complexes. There were two special positions found: the N-terminal end of αCtx lacking two residues contrast with the end of αBgt. When αBgt-HAP form five hydrogen bonds, αCtx–HAP can only form one; Arg2 of HAP forms the H-bond and salt bridge with αBgt residue Asp30, while a huge side chain of Arg36 forming intramolecular contacts with Asp27 prevents the formation of contact with similar αCtx residue Asp27 [4].

To solve this problem, researchers have found a version of HAP whose peptide only have a single residue change – Leu9Glu – and form an additional H-bond and a salt bridge with α-neurotoxins. The diagram (Fig.4 and Fig.5) shows that the HAP has a high affinity of αBgt, with the Kd of 21~37, the change of HAP on L9E will improve the affinity of the combination of αCtx. Even they have found that αBgt demonstrated a higher affinity for both HAP and HAP[L9E] as compared to αCtx, it is shown that the HAP[L9E] peptide binds to αCtx has more affinity [5].

3. Synthesis of HAPs with strong α-BTX inhibitory potency

In the study of the treatment of α-BTX poisoning, the synthesis of high affinity peptide (HAP) has made great progress in protecting α-BTX toxicity and mortality. In this review, general methods and ideas for the design and synthesis of α-BTX binding peptides that inhibit their binding to nACHR are introduced.

In order to study the binding site of α-BTX on nicotinic acetylcholine receptors, appropriate mimotope peptides of these nAChRs are required, and thus, HAP was found [6]. In the research conducted by E. Katchalski-Katzir et al., a combinatorial phage-display peptide library that contained 15 residues was employed and a 13-mer peptide (MRYYESSLKSYPD) that binds to α-BTX specifically was identified; same research had also found that peptides with residues YYXSSL bind to α-BTX with high affinity as lead peptide for further modification [5,6]. This peptide and its analogs had been studied as classical HAPs to α-BTX.

The binding of the lead peptide to α-BTX shows high irreversibility and specificity [7], in order to determine the important residue interactions that contribute to these features, chimeric analysis of toxin-peptide complex should be carried out. Before the further structure analysis of α-BTX-lead peptide complex had been conducted, peptides bearing YYXSSL residue sequence were found to bind with α-BTX with high affinity, and the binding with α-BTX was found requires two adjacent aromatic

residues [8]. In the chimeric analysis studies, X-Ray treatment and NMR are commonly used techniques in visualizing the crystalline structure of α-BTX-lead peptide complex [7]. The binding characteristic of lead peptide to α-BTX was thoroughly studied by applying NMR technique, the residues of the lead peptide (MRYYESSLKSYPD) that interact with the α-BTX are Arg2, Tyr3, Tyr4, Glu5, Ser7, Leu8 and Tyr11 (see Tab 1 and Fig 1), other residues (Met1, Ser6, Lys9, Ser10, Pro12 and Asp13) have no detectable contribution to the binding [8,9]. The binding ability of leading peptide to α-BTX was found to be greatly enhanced by systematic amino acid residue replacement on the positions 1, 6, 9, 10, 12 and 13 [9].

Table 1. Intermolecular NMR-observed interactions and contact areas of the library-lead peptide in their corresponding complexes with α-BTX [8].

Library-lead peptide			
Peptide residues	Interacting α-BTX residues	No. of interactions	Contact area, Å^2
M1	-	-	-
R2	V39	1	46
Y3	T6, A7, I11, V39, H68	17	135
Y4	D30, R36, V39, V40	21	125
E5	K38, V39, V40, H68	8	104
S6	-	-	-
S7	H68, R72	3	32
L8	I11, H68, K70, Q71	14	97
Y11	-	-	-

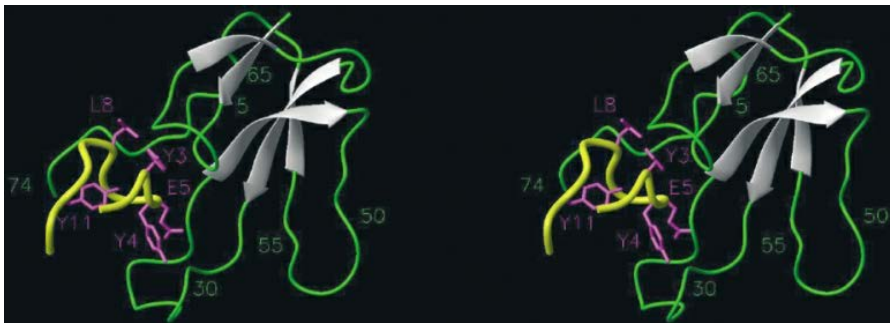

Figure 1. Stereoscopic view of the complex of α-BTX with the library-lead peptide as determined by NMR spectroscopy. α-BTX residues amare shown in gray (b-sheet regions) and green, and the peptide backbone is shown in yellow. Side chains of peptide residues interacting with the toxin (Y3, Y4, E5 and L8), and forming the hydrophobic core of the peptide (Y11), are shown in pink. [5]

Systematic single residue replacement of the lead peptide was successfully carried out and generated 38 different peptides, their inhibitory activities (IC_{50}) were measured (Table 2). As shown in Table 2, most modified peptides stay same or even a greater inhibitory activity that that lead peptide (ranged from 1×10^{-7} to 3×10^{-7} M). Some peptides exhibited lower or no inhibitory activity to α-BTX. Peptide No. 29 (which background color is marked grey in Table 2) showed one magnitude higher inhibitory activity than that lead peptide [9,10]. The possible explanation of increased affinity was that the substitution of Ser10 by Pro mimicked the Proline subunit of nAChR of those α-BTX sensitive species, which contributes to α-BTX binding.

Table 2. Inhibition of the binding of α-BTX to Torpedo AChRs by synthesized peptides, prepared by systematic single replacement of the library-lead peptide [9]. The replaced amino acid residues are underlined and bold.

Peptides	Position													IC_{50} (M)
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Library	M	R	Y	Y	E	S	S	L	K	S	Y	P	D	3.3×10^{-7}
1	**L**	R	Y	Y	E	S	S	L	K	S	Y	P	D	2.9×10^{-7}
2	**Y**	R	Y	Y	E	S	S	L	K	S	Y	P	D	1.0×10^{-6}
3	**K**	R	Y	Y	E	S	S	L	K	S	Y	P	D	5.0×10^{-6}
4	**D**	R	Y	Y	E	S	S	L	K	S	Y	P	D	5.0×10^{-6}
5	**G**	R	Y	Y	E	S	S	L	K	S	Y	P	D	5.0×10^{-6}
6	**P**	R	Y	Y	E	S	S	L	K	S	Y	P	D	1.5×10^{-7}
7	M	R	Y	Y	E	**L**	S	L	K	S	Y	P	D	5.0×10^{-6}
8	M	R	Y	Y	E	**Y**	S	L	K	S	Y	P	D	1.1×10^{-7}
9	M	R	Y	Y	E	**K**	S	L	K	S	Y	P	D	5.0×10^{-6}
10	M	R	Y	Y	E	**D**	S	L	K	S	Y	P	D	No inhibition
11	M	R	Y	Y	E	**T**	S	L	K	S	Y	P	D	5.0×10^{-6}
12	M	R	Y	Y	E	**G**	S	L	K	S	Y	P	D	5.0×10^{-6}
13	M	R	Y	Y	E	**P**	S	L	K	S	Y	P	D	No inhibition
14	M	R	Y	Y	E	S	**T**	L	K	S	Y	P	D	1.0×10^{-5}
15	M	R	Y	Y	E	S	**Y**	L	K	S	Y	P	D	No inhibition
16	M	R	Y	Y	E	S	**N**	L	K	S	Y	P	D	1.0×10^{-5}
17	M	R	Y	Y	E	S	**S**	L	**L**	S	Y	P	D	3.3×10^{-7}
18	M	R	Y	Y	E	S	S	L	**F**	S	Y	P	D	5.0×10^{-6}
19	M	R	Y	Y	E	S	S	L	**R**	S	Y	P	D	1.0×10^{-6}
20	M	R	Y	Y	E	S	S	L	**D**	S	Y	P	D	1.5×10^{-7}
21	M	R	Y	Y	E	S	S	L	**S**	S	Y	P	D	5.0×10^{-6}
22	M	R	Y	Y	E	S	S	L	**G**	S	Y	P	D	5.0×10^{-6}
23	M	R	Y	Y	E	S	S	L	**P**	S	Y	P	D	No inhibition
24	M	R	Y	Y	E	S	S	L	K	**L**	Y	P	D	1.0×10^{-5}
25	M	R	Y	Y	E	S	S	L	K	**Y**	Y	P	D	5.0×10^{-6}
26	M	R	Y	Y	E	S	S	L	K	**K**	Y	P	D	1.0×10^{-5}
27	M	R	Y	Y	E	S	S	L	K	**D**	Y	P	D	5.0×10^{-6}
28	M	R	Y	Y	E	S	S	L	K	**G**	Y	P	D	2.1×10^{-7}
29	M	R	Y	Y	E	S	S	L	K	**P**	Y	P	D	3.2×10^{-8}
30	M	R	Y	Y	E	S	S	L	K	S	Y	**L**	D	5.0×10^{-6}
31	M	R	Y	Y	E	S	S	L	K	S	Y	**Y**	D	5.0×10^{-6}
32	M	R	Y	Y	E	S	S	L	K	S	Y	**K**	D	5.0×10^{-6}
33	M	R	Y	Y	E	S	S	L	K	S	Y	**D**	D	5.0×10^{-6}
34	M	R	Y	Y	E	S	S	L	K	S	Y	**G**	D	5.0×10^{-6}
35	M	R	Y	Y	E	S	S	L	K	S	Y	P	**F**	5.0×10^{-6}
36	M	R	Y	Y	E	S	S	L	K	S	Y	P	**K**	5.0×10^{-5}
37	M	R	Y	Y	E	S	S	L	K	S	Y	P	**E**	1.4×10^{-7}
38	M	R	Y	Y	E	S	S	L	K	S	Y	P	**G**	5.0×10^{-6}

Multiple studied had focused on the comparison of the amino acid sequences of nAChR ligand-binding sites found in different species with varied sensitivity towards α-BTX [11,12] (see Table 3), Trp187 of nAChR was found essential for α-BTX binding, and this residue presented in the nAChR of α-BTX sensitive species (Torpedo, chicken, mouse), but in the nAChR of α-BTX resistant species (snake, mongoose), position 187 was not tryptophan. Based on these available biological data, Kasher and coworkers synthesized additional peptide series by single residue replacement [9,10], including

peptide in which Met1 is replaced by Trp (peptide No. 39, the background color is marked grey in Table 4) that mimicked the aromatic subunit of nAChR, which showed IC_{50} value of 3.5×10^{-8}M, one order of magnitude higher than the inhibitory activity of lead peptide.

Table 3. Alignment of amino acid sequence of the library-lead peptide in comparison with the sequence of the α-BTX-binding region of muscle AChR (position 187 to 200 in the K-subunit) from different animal species [11].

α-BTX binding	Source	187		189			192	193	194			197		199	200
++	*Torpedo*	**W**	V	**Y**	**Y**	T	C	C	**P**	D	T	**P**	Y	L	D
++	Chicken	**W**	.	**Y**	**Y**	A	.	.	**P**	.	.	**P**	.	.	.
++	Mouse	**W**	.	**F**	**Y**	S	.	.	**P**	T	.	**P**	.	.	.
+	Human	S	.	T	**Y**	S	.	.	**P**	.	.	**P**	.	.	.
-	Hedgehog	R	.	I	**Y**	A	.	.	**P**	S	.	**P**	.	.	.
-	Cobra	S	.	N	**Y**	S	.	.	L	.	.	**P**	.	.	.
-	Mongoose	N	.	T	**Y**	A	.	.	L	T	.	H	.	.	.

Table 4. Inhibition of binding of α-BTX to Torpedo AChR by synthesized peptides designed on the basis of biological information [10]. Amino acid residues that were replaced are underlined and bold.

Peptides	**Position**													IC_{50} (M)
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Library	M	R	Y	Y	E	S	S	L	K	S	Y	P	D	3.3×10^{-7}
39	**W**	R	Y	Y	E	S	S	L	K	S	Y	P	D	3.5×10^{-8}
40	M	**V**	Y	Y	E	S	S	L	K	S	Y	P	D	5.0×10^{-6}
41	M	R	**F**	Y	E	S	S	L	K	S	Y	P	D	5.0×10^{-6}
42	M	R	**W**	Y	E	S	S	L	K	S	Y	P	D	No inhibition
43	M	R	Y	Y	E	S	S	**P**	K	S	Y	P	D	5.0×10^{-5}
44	M	R	Y	Y	E	S	S	**E**	K	S	Y	P	D	5.0×10^{-5}
45	M	R	Y	Y	E	S	S	**K**	K	S	Y	P	D	No inhibition
46	M	R	Y	Y	E	S	S	**P**	**T**	**T**	**P**	**Y**	**L**	No inhibition
47	**W**	**V**	**F**	Y	**S**	**C**	**C**	L	K	S	Y	P	D	No inhibition
Torpedo	W	V	Y	Y	T	C	C	P	D	T	P	Y	L	-

To reinforce the inhibiting effect of the peptide, Kasher and co-workers combined the substitutions done on peptide 29 and 39, which performed considerably higher inhibitory activity, and conducted multiple residues replacement. This study generated high-affinity peptides with inhibitory potency that was stronger than original lead peptide by two orders of magnitude [9,10] (Table 5), which were peptide No. 50, No. 52, No. 53, No. 54, and No. 56 (which the background color was marked grey in Table 5).

Table 5. Inhibition of binding of α-BTX to AChR by synthesized peptides, obtained by multiple amino-acid residue substitutions of the library-lead peptide [9]. Amino acid residues that were replaced are underlined and bold.

Peptides	Position													IC_{50} (M)
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Library	M	R	Y	Y	E	S	S	L	K	S	Y	P	D	3.3×10^{-7}
48	**W**	R	Y	Y	E	S	S	L	K	**P**	Y	P	D	1.0×10^{-8}
49	**W**	R	Y	Y	E	S	S	L	**D**	**P**	Y	P	D	3.8×10^{-8}
50	**W**	R	Y	Y	E	S	S	L	**E**	**P**	Y	P	D	2.0×10^{-9}
51	**W**	R	Y	Y	E	S	S	**K**	**E**	**P**	Y	P	D	5.8×10^{-8}
52	**W**	R	Y	Y	E	**Y**	S	L	**D**	**P**	Y	P	D	1.6×10^{-9}
53	**W**	R	Y	Y	E	S	S	L	**D**	**P**	Y	P	**E**	4.8×10^{-9}
54	**W**	R	Y	Y	E	S	S	L	**L**	**P**	Y	P	D	1.9×10^{-9}
55	M	R	Y	Y	E	**C**	**C**	L	K	S	Y	P	D	3.3×10^{-8}
56	**W**	R	Y	Y	E	**C**	**C**	L	**D**	**P**	Y	P	D	1.9×10^{-9}

4. Database of HAP

Based on the work described above, a database about HAP which can replace the nACHR has been developed. A great many works can be summarized into a library, which would facilitate searching and obtaining the required information. Thus, it is urgent to form the further development with our library.

The first problem researchers shall face is the different sequences with a large amount, especially for those high-throughput screening to select the peptides with biological activity. With the method of panning and screening, the significant peptides would be introduced into the library. Next step would be to combine the data from the databases and make it a new potential targeted drug [13]. For example, LLP, the origin of the peptide can lead the future design of new bioactive peptides which more effective. The design of HAP is based on the library of LLP. Researchers first looked for the amino acid which may combine with α-neurotoxin from the database, and found out amino acids which cannot be replaced and change the others which will never affect the structure of peptide.

A reasonable approach may make the first step of a new design. New design can refer to the peculiarity of the library which they have designed before, and get the train of thought from another direction. Scientists have achieved the synthesis of longer peptides and shorter peptides. Also, the library of LLP has been extensively used.

Table 6. The binding inhibition caused by the structure, which is listed as the library numbered by the codes [14].

Code	Structure	% Binding inhibition
L1	cyclo(Xxx-Xxx-Xxx-$_L$-Pro-$_D$-Asp)	0
L2	cyclo(Xxx-Xxx-Xxx-$_L$-Val-$_D$-Asp)	0
L3	cyclo(Xxx-Xxx-Xxx-$_L$-Leu-$_D$-Asp)	0
L4	cyclo(Xxx-Xxx-Xxx-$_L$-Trp-$_D$-Asp)	0
L5	cyclo(Xxx-Xxx-Xxx-$_L$-Arg-$_D$-Asp)	4.0
L6	cyclo(Xxx-Xxx-Xxx-$_L$-Glu-$_D$-Asp)	7.7
L7	cyclo(Xxx-Xxx-Xxx-$_D$-Pro-$_D$-Asp)	0
L8	cyclo(Xxx-Xxx-Xxx-$_D$-Val-$_D$-Asp)	0
L9	cyclo(Xxx-Xxx-Xxx-$_D$-Leu-$_D$-Asp)	0
L10	cyclo(Xxx-Xxx-Xxx-$_D$-Trp-$_D$-Asp)	72.3
L11	cyclo(Xxx-Xxx-Xxx-$_D$-Arg-$_D$-Asp)	0
L12	cyclo(Xxx-Xxx-Xxx-$_D$-Glu-$_D$-Asp)	0
L13	cyclo(Xxx-Xxx-$_L$-Pro-Xxx-$_D$-Asp)	0
L14	cyclo(Xxx-Xxx-$_L$-Val-Xxx-$_D$-Asp)	0
L15	cyclo(Xxx-Xxx-$_L$-Leu-Xxx-$_D$-Asp)	76.7
L16	cyclo(Xxx-Xxx-$_L$-Trp-Xxx-$_D$-Asp)	45.4
L17	cyclo(Xxx-Xxx-$_L$-Arg-Xxx-$_D$-Asp)	15.1
L18	cyclo(Xxx-Xxx-$_L$-Glu-Xxx-$_D$-Asp)	1.2
L19	cyclo(Xxx-Xxx-$_D$-Pro-Xxx-$_D$-Asp)	0
L20	cyclo(Xxx-Xxx-$_D$-Val-Xxx-$_D$-Asp)	0
L21	cyclo(Xxx-Xxx-$_D$-Leu-Xxx-$_D$-Asp)	0
L22	cyclo(Xxx-Xxx-$_D$-Trp-Xxx-$_D$-Asp)	14.8
L23	cyclo(Xxx-Xxx-$_D$-Arg-Xxx-$_D$-Asp)	0
L24	cyclo(Xxx-Xxx-$_D$-Glu-Xxx-$_D$-Asp)	0
L25	cyclo(Xxx-$_L$-Pro-Xxx-Xxx-$_D$-Asp)	10.8
L26	cyclo(Xxx-$_L$-Val-Xxx-Xxx-$_D$-Asp)	11.6
L27	cyclo(Xxx-$_L$-Leu-Xxx-Xxx-$_D$-Asp)	7.0
L28	cyclo(Xxx-$_L$-Trp-Xxx-Xxx-$_D$-Asp)	3.0
L29	cyclo(Xxx-$_L$-Arg-Xxx-Xxx-$_D$-Asp)	0.3
L30	cyclo(Xxx-$_L$-Glu-Xxx-Xxx-$_D$-Asp)	0
L31	cyclo(Xxx-$_D$-Pro-Xxx-Xxx-$_D$-Asp)	31.2
L32	cyclo(Xxx-$_D$-Val-Xxx-Xxx-$_D$-Asp)	37.3
L33	cyclo(Xxx-$_D$-Leu-Xxx-Xxx-$_D$-Asp)	11.5
L34	cyclo(Xxx-$_D$-Trp-Xxx-Xxx-$_D$-Asp)	4.9
L35	cyclo(Xxx-$_D$-Arg-Xxx-Xxx-$_D$-Asp)	0
L36	cyclo(Xxx-$_D$-Glu-Xxx-Xxx-$_D$-Asp)	8,5
L37	cyclo($_L$-Pro-Xxx-Xxx-Xxx-$_D$-Asp)	43.6
L38	cyclo($_L$-Val-Xxx-Xxx-Xxx-$_D$-Asp)	0
L39	cyclo($_L$-Leu-Xxx-Xxx-Xxx-$_D$-Asp)	0
L40	cyclo($_L$-Trp-Xxx-Xxx-Xxx-$_D$-Asp)	0
L41	cyclo($_L$-Arg-Xxx-Xxx-Xxx-$_D$-Asp)	0
L42	cyclo($_L$-Glu-Xxx-Xxx-Xxx-$_D$-Asp)	0
L43	cyclo($_D$-Pro-Xxx-Xxx-Xxx-$_D$-Asp)	8.4
L44	cyclo($_D$-Val-Xxx-Xxx-Xxx-$_D$-Asp)	0
L45	cyclo($_D$-Leu-Xxx-Xxx-Xxx-$_D$-Asp)	0
L46	cyclo($_D$-Trp-Xxx-Xxx-Xxx-$_D$-Asp)	10.3
L47	cyclo($_D$-Arg-Xxx-Xxx-Xxx-$_D$-Asp)	15.2
L48	cyclo($_D$-Glu-Xxx-Xxx-Xxx-$_D$-Asp)	0

The preparatory work of using library includes not only the pre-analysis of the structure, but also the application of the peptide. If the peptide has no effect based on the theoretical analysis, the library would not be accepted as well.

A good leading compound should be then chosen for further steps. The three-dimensional structure, critical positions and residues are the most important information in peptide study. Molecular modeling can help develop new peptide, and some library would provide the ability to generate higher affinity and make them more structural constraints. A typical example is the biological degradation, in another words, combining to the α-neurotoxin and making them useless [13]. In the future, an increasing number of libraries would be developed to support related studies.

5. Chemically modified peptides

Researchers have used powerful technologies to create large libraries for DNA-encoded peptide and protein. To form that, researchers may synthetic DNA from the transcription from mRNA and choose the expressed peptides and screen them by their biological performance. Connection between useful peptides and encoding DNA can be found, and isolated by Darwinian selection process. However, all of the protein existed in our body is combined with no more than 20 amino acids. Furthermore, the side chains of amino acids made with chemical groups are also restrict the space for the limited groups. However, the development of small peptides of pharmaceutical leads is more difficult than small molecule chemistry.

In solving these problems, researchers have been bringing in chemical modification. Molecule chemistry is usually enormous, and it is urgent to divert these conditions as expected. Importantly, the peptide and proteins can enlarge their libraries. When introduce these modification, more potent binding affinities and better proteolytic stabilities would be accepted by the peptides.

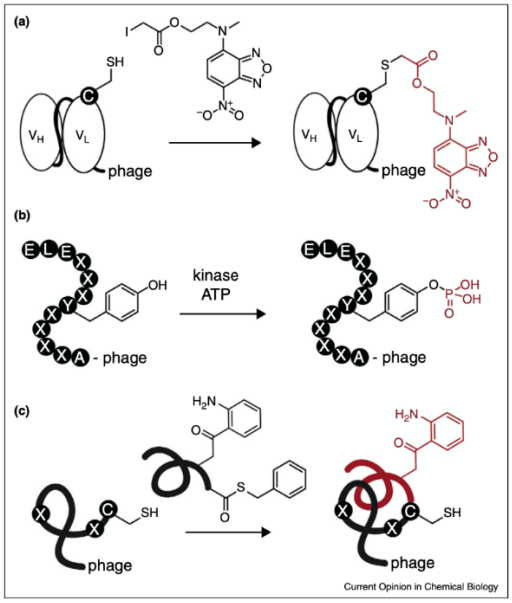

Figure 2. Non-reducible cross-linkers have been used to generate both monocyclic and bicyclic ligands, such constraints leading to more potent binding affinities and better proteolytic stabilities [15].

Structures have been separated into several types: simple peptide conjugates, monocyclic peptides, bicyclic peptides.

5.1 Simple Peptide Conjugates

Researchers combine small chemical entities to peptide libraries, in this way, they expand the chemical diversity of the libraries [16]. They first propose the strategy that they conjugate pharmacophores which they have proved to DNA-encoded peptide libraries, and they pick the high affinity peptides within them. Similar chemistry was used to build a model pharmacophore [17].

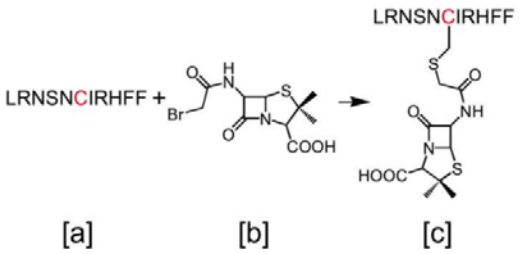

Figure 3. Researchers proved their strategy and give out the consequence that they conjugated a penicillin derivative of iodoacetamide to an invariant cysteine in an mRNA-encoded peptide library. In this library, they identify that penicillin-peptide conjugates bounds better than penicillin [17].

5.2 Monocyclic Peptides

Researchers improved their chemical bioactivity by decorate the structure of the peptides and found cyclic peptides bind higher affinity to proteins, which only cost a smaller entropy than simple conjugates. Importantly, their proteolytic stability also gives an excellent environment for treatments [18,19].

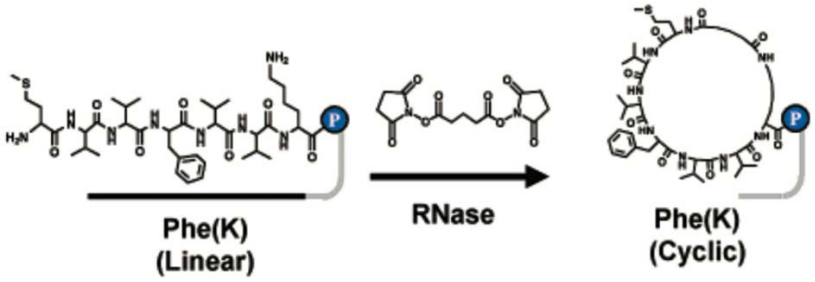

Figure 4. In order to produce irreducible cyclic peptide ligands, researchers cyclized the encoded peptides with chemical linkers formed by amide bonds. It was demonstrated that the peptide encoded by mRNA can be linked to the N-terminal of the peptide by using disuccinyl glutarate (DSG) α Amino and lysine residues ε Amine [20].

5.3 Bicyclic Peptides

The conformation of monocyclic peptides has a single bond to limit the flexibility of the peptide main chain, while bicyclic peptides have two bonds and are expected to be more rigid. Two macrocycles may form contact with protein targets at the same time, so as to interact closely [21,22].

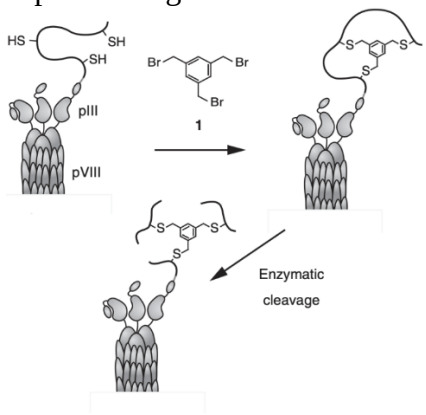

Figure 5. Researchers used the small organic compound tris-(bromomethyl) benzene as a scaffold to anchor peptides containing three cysteine residues, and the threefold rotational symmetry of the TBMB molecule ensures the formation of a unique structural and spatial isomer [21].

6. Conclusion

An increasing number of novel type of neurotoxins with structurally and functionally features has been discovered, and the database of snake toxins has been expanded. The design of HAP clarifies the

orientation of the future cure of α-neurontoxins. Thus, in this way, it may provide other imaginations to envisage ambitious strategies for protein and genetic engineering and design of molecular probes.

Polypeptide is a molecule with a wide application prospect. Compared with antibodies and other macromolecules, they are smaller and can enhance the permeability of tissues. These polypeptides easily prepared by chemical synthesis and can be easily modified and connected to different functional parts. Through the combination of biological or synthetic peptide library and reasonable molecular design, researchers can produce molecular diversity and select bioactive peptides for a variety of biological targets. The combination of the two methods can occur in different steps of the selection process. According to the availability of structural and functional information about peptides and their targets, it can be easily modified and linked to functional positions.

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