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Design and characterization of peptides that neutralize the venom of the snake *Bungarus multicinctus*

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In our laboratory we employ a combinatorial phage-display peptide library as a powerful tool for searching peptide molecules that match any desired target molecules. Small and stable protein molecules, such as snake toxins, were used as model systems to study protein-protein interactions. We describe here a successful detection of two library-selected peptides that bind and neutralize the toxicity of two different neurotoxins in the venom of the snake *Bungarus multicinctus*. Following the snake bite, α-BTX (α-bungarotoxin) and β-BTX work in concert to cause peripheral muscle failure and death. Both toxins are directed to the peripheral neuromuscular junction. α-BTX binds to the nicotinic acetylcholine receptor (AChR), consequently blocking the opening of the receptor channel at the post-synaptic membrane. β-BTX is a dimer composed of chain A, a phospholipase A2 subunit, and chain B, a subunit that binds certain voltage-gated potassium channels at the pre-synaptic membrane. Targeting chain A to the potassium channel facilitates destruction of the membrane by the enzyme, resulting in the blocking of acetylcholine release.

In collaboration with Sara Fuchs, we screened a phage-display peptide library and isolated a lead peptide (MRYYESSLKSYPD) that binds α-BTX and inhibits its binding to AChR with a moderate potency (10^{-6}M). Alignment of the consensus sequence (YYXSS) of the library-selected peptides with AChRs revealed that this sequence mimics the motif YYXCC, present at positions 189-193 of the AChRs α-subunit, in animal species that are sensitive to α-BTX (Balass et al., 1997). In a further study in collaboration with Jacob Anglister and Tali Scherf using an NMR technique, the structure of the lead peptide in complex with α-BTX was solved (Scherf et al., 1997). Based on the NMR structural data, a new cyclic peptide (CRYYESSLKSYCD), which forms a disulfide bond following the oxidation of the thiol groups on cysteines 1 and 12, was generated. The cyclic peptide binds to α-BTX with an affinity two order of magnitudes higher than that of the lead peptide. The cyclic peptide confers full protection against α-BTX lethality in mice (Balass et al., 2001).

The affinity of the lead peptide was highly improved by using our novel technique, designated SRR (Systematic Residue Replacement). The SRR approach enabled us to generate four types of high affinity peptides (HAP1-HAP4) that bind to the toxin with an affinity of about 10^{-9}M (Kasher et al., 2001). The three dimensional structure of HAP1 (WRYYESSLEPYPD) in complex with the toxin was further studied by our colleague Tali Scherf, using the NMR technique [Fig. 1] (Scherf et al., 2001). In addition, the crystal structure of another high-affinity peptide, HAP2 (WRYYESSLLPYPD) in complex with the toxin, was analyzed by x-ray diffraction at 1.8Å resolution by our colleagues Joel Sussman, Michal Harel and their collaborators (Harel et al., 2001). The results obtained from the above two techniques indicate that both HAP1 and HAP2 in complex with the toxin, assume a β-hairpin folded structure. This structure is formed by two antiparallel β-strands, existing in both peptides. Remarkably, it resembles the homologous loop of acetylcholine-binding protein, an analogue of the soluble extracellular domain of AChR α-subunit (Brejc et al., Nature 411:269, 2001). Since α-BTX competes with acetylcholine for the AChR, it was suggested that both are targeted to overlapping sites.

We recently detected a library-peptide (CAEVSTWEM-LQQLNTTRMPPPC) that binds β-BTX with an affinity of 10^{-6}M. This peptide is directed to the phospholipase A2 subunit of the toxin, and upon binding it neutralizes its enzymatic activity.

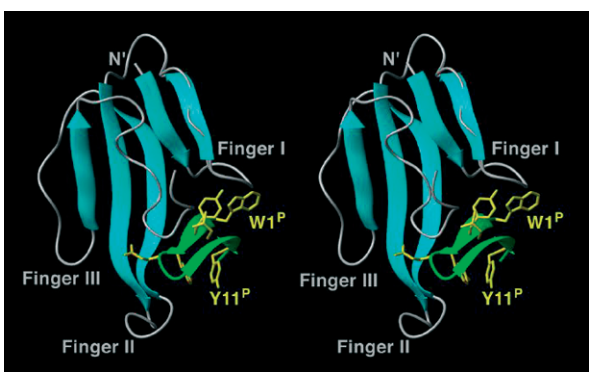

Fig. 1 *Three dimensional solution structure of the high affinity peptide (HAP1, WRYYESSLEPYPD) in complex with α-BTX (Scherf et al.). The ribbon diagram of the α-BTX backbone is shown in cyan (β-sheet regions) and in gray. Peptide backbone is shown in green, and side chains of W1,Y3, Y4, E5, L8 and Y11 are shown in yellow. The figure was generated by Tali Scherf.*

Preliminary results indicate that in a similar manner to the α-BTX-directed peptides, this peptide also confers full protection from mice lethality caused by the corresponding toxin. It is thus assumed that a mixture of the above synthetic peptides will neutralize the whole venom of the B. multicinctus. The approach described above might be used to develop peptide-antidotes for any venom that contains one or more toxic components.

Selected Publications

Balass, M., Katchalski-Katzir, E. and Fuchs, S. (1997) The α-bungarotoxin binding site on the nicotinic acetylcholine receptor: analysis using a phage-epitope library. Proc. Natl. Acad. Sci. USA 94, 6054-6058.

Scherf, T., Balass, M., Fuchs, S., Katchalski-Katzir, E. and Anglister, J. (1997) Three-dimensional solution structure of the complex of α-bungarotoxin with a library-derived peptide. Proc. Natl. Acad. Sci. USA 94, 6059-6064.

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Kasher, R., Balass, M., Scherf, T., Fridkin, M., Fuchs, S. and Katchalski-Katzir, E. (2001) Design and synthesis of peptides that bind α-bungarotoxin with high affinity. Chemistry and Biology 8, 147-155.

Scherf, T., Kasher, R., Balass, M., Fridkin, M., Fuchs, S., and Katchalski-Katzir, E. (2001) A β-hairpin structure in a 13-mer peptide that binds α-bungarotoxin with high-affinity and neutralizes its toxicity. Proc. Natl. Acad. Sci. USA 95 , 6629-6634.

Harel, M., Kasher, R., Nicolas, AJ., Guss, M., Balass, M., Fridkin, M., Smit, AB., Brejc, K., Sixma, T., Katchalski-Katzir E., Sussman, JL. and Fuchs, S. (2001) The structure of the binding site of the acetylcholine receptor as visualized in the X-ray structure of a complex between α-bungarotoxin and a high affinity mimotope peptide. Neuron 32, 265-275.