Design and Characterization of Polyvalent Anthrax Toxin Inhibitors

by

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ABSTRACT

Polyvalency – the simultaneous binding of multiple ligands on one biological entity to multiple receptors on another – is a phenomenon that is commonly found in nature. We are using a biomimetic approach, inspired by polyvalency, to design potent inhibitors of anthrax toxin. Since the major symptoms and death from anthrax are due primarily to the action of anthrax toxin, the toxin is a prime target for therapeutic intervention. We describe the design of potent liposome-based anthrax toxin inhibitors, and demonstrate that statistical pattern matching enhances the potency of these inhibitors. We functionalized liposomes with an inhibitory peptide at different densities and observed a transition in potency at an inter-peptide separation that matches the distance between ligand-binding sites on the heptameric component of anthrax toxin. Pattern-matched polyvalent liposomes inhibited anthrax toxin *in vitro* at concentrations four orders of magnitude lower than the corresponding monovalent peptide, and neutralized this toxin *in vivo*. Furthermore, the phenomenon of statistical pattern matching was not unique to anthrax toxin; it also facilitated the inhibition of cholera toxin by galactose-functionalized liposomes. We have also designed biomimetic inhibitors inspired by lipid rafts, as well as inhibitors that target the cellular receptor for anthrax toxin. These biomimetic anthrax toxin inhibitors may enable the successful treatment of anthrax during the later stages of the disease when antibiotic treatment is ineffective. Designing antimicrobial therapeutics that target host cell proteins used by pathogens to cause the disease represents a promising strategy to overcome the growing problems associated with antibiotic drug resistance. We also describe the design of phase separated PEG liposome based polyvalent inhibitors of anthrax toxin that are not only as potent as corresponding conventional liposomes but also exhibit improved colloidal stability therapy improving the storage stability of these liposomes. Attaching a polymer like PEG to the surface of liposomes is also known to improve the *in vivo* circulation lifetimes of these liposomes. Further we have designed polyvalent inhibitors based on a peptide composed of all d-amino acids and this represents a major improvement in our continued quest towards the development of a polyvalent therapeutic against anthrax.

We have also carried out studies that characterize the binding interactions between a peptide ligand and the anthrax toxin receptor by using biomolecular NMR techniques.

Our approach to designing and characterizing polyvalent anthrax toxin inhibitors described in this thesis report should be broadly applicable to a variety of other pathogens, viruses, bacteria and their toxins. It will be especially important to apply all the concepts involved in this work to diseases where alternative treatments are gravely needed, which include diseases such as influenza, AIDS, and malaria.