The Interaction of Cytotoxins with a Lipid Membrane Library

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Abstract:

Cytotoxins are agents toxic to cells. To infect, cytotoxins must overcome the cell membrane, the primary defense of the cell. Membranes, however, are highly heterogeneous, containing many distinct domains differing by lipid content. For most cytotoxins, it is unknown if individual species differentiate between lipid compositions or if some domains act as nucleation sites for aggregation. Using microfluidic techniques, we studied the binding affinity of inert Amyloid-Beta 40 and toxic Amyloid-Beta 42, a primary suspect that exhibits neurotoxic activity leading to Alzheimer's dementia, and the membrane binding portion of the anthrax toxin, to a variety of lipid domains. By introducing the cytotoxins to a lipid domain library we were able to examine their binding propensities to lipid domains; we find this to be a selective process.

Introduction:

Bio-membranes are composed of thousands of lipid species, differing in their alkyl chains, headgroups and degree of saturation. Changes in lipid composition or even the absence of a single lipid have shown to lead to severe pathologies and death.

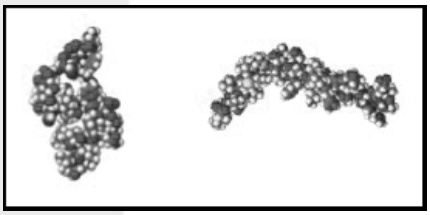

Figure 1: The inert amyloid-beta 40 peptide (left) and the toxic amyloid-beta 42 peptide (right). (See full color version on page xxxvi.)

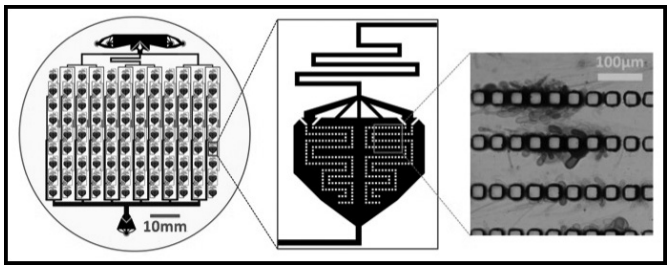

Figure 2: Magnifying from left to right; The 108 well microfluidic device; a single well; individual liposome swelling within the well.

The leading hypothesis that explains the role of lipids in membrane functionality is that the lipids segregate into distinct domains [1]. These lipid domains can, with high specificity, incorporate or exclude proteins, hence inhibiting or accelerating biological processes at the membrane surface. Structural studies of lipid membranes have shown that the lipid packing, distances and tilt, strongly depend on the their chain length, backbone, and headgroup. This suggests that lipid complexes may have structural and chemical complementarities with proteins [2]. Knowledge of protein-domain interactions is essential to understand membrane functionality.

The complementation of lipid domains with specific proteins suggests specific binding patterns of lipid membranes with various pathogens. Our research examined the interaction of amyloid-beta (Aβ) peptides and the anthrax toxin with various lipid domains. Aβ peptides, originally the intermembrane component of the amyloid precursor protein, are found in high concentrations in the brains of Alzheimer's patients. $A\beta_{40}$ and $A\beta_{42}$ (Figure 1) are the two dominant forms of the Aβ peptide. Due to its more hydrophobic nature, the $A\beta_{42}$ is the most amyloidogenic (and fibrillogenic) form of the peptide and considered the primary toxin in Alzheimer's. Using microfluidic techniques, we examined the affinity of $A\beta_{40}$ and $A\beta_{42}$ peptides to a lipid membrane library. In parallel with this experiment, and using similar techniques, we studied the binding of the anthrax toxin to the lipid library.

Experimental Procedure:

Using the largest lipid library in the world, consisting of 108 different lipid domains, we analyzed the binding selectivities of cytotoxins. We used a self-designed and fabricated 108 well polydimethylsiloxane (PDMS) microfluidic device (Figure 2) to introduce cytotoxins to the lipid library. The device gave

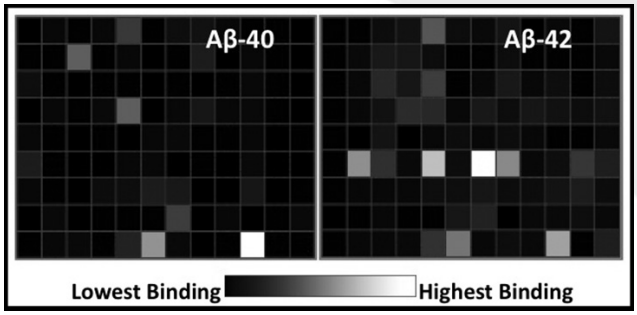

Figure 3: Amyloid-beta affinity matrices; Each square corresponds spatially to the wells on the microfluidic device. Note the distinct variations in the binding specificities the two amyloid-beta peptides.

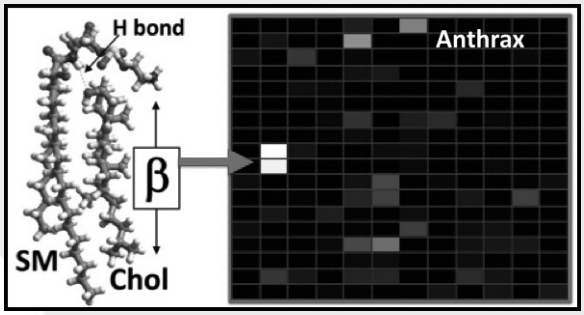

Figure 4: Anthrax affinity matrix; The toxin demonstrates high selectivity to sphingomyelin-cholesterol complexes shown left.

us tight control over small volumes, allowing us to analyze multiple samples in parallel. Liposomes were grown inside microfluidic channels by hydrating and heating lipids that were seeded within the device during fabrication. After liposome formation, fluorescently labeled cytotoxins were flushed into the device and allowed to interact with the liposomes. The liposomes were then washed and unbound cytotoxins were flushed out of the device. Using confocal microscopy and extensive image analysis, we calculated the total fluorescence per unit area of the liposomes for each well. From this information, we were able to identify the lipid domains to which the cytotoxins were bound.

Results and Conclusions:

Using microfluidics, we analyzed, in parallel, the binding preferences of a large number of lipid domains, and created affinity matrices that showed the specific binding of Aβ peptides and anthrax toxin to lipid domains. These matrices show the relative fluorescence per unit area for each lipid domain. The different binding patterns of $A\beta_{40}$ and $A\beta_{42}$ (Figure 3) reveal variation in membrane binding propensities, and hence, possible differences in cytotoxicity. The binding of the peptides to cell membranes is considered the toxic step in Alzheimer's disease due to the theory that bound peptides induce neuron membrane permeability and plaque formation in the brains of Alzheimer's patients. This in mind, it is important to note that $A\beta_{42}$, considered the more toxic peptide, bound favorably to lipid domains containing cerebrosides, lipids found abundantly in the surface membranes of neural cells, while $A\beta_{40}$ bound favorably to the domains containing phosphatidylethanolamine, lipids rarely found on the surface of neural cells.

We also created an affinity matrix for the binding pattern of the anthrax toxin (Figure 4). A series of experiments showed that the toxin had high selectivity to sphingomyelin-cholesterol complexes; these domains are considered very important for membrane functionality. This selective binding may be a useful tool, allowing scientists, for the first time, to use anthrax as a probe to label specific lipid domains.

Future Work:

To further this work, the affinity of cytotoxins to various cell lines would need to be tested. Liposomes are model membranes; the model must be proven by showing the binding selectivity of cytotoxins to various cell membranes of unique lipid compositions. Work of this nature would shed light on the toxic mechanism of Alzheimer's disease and the possibility of using anthrax as a probe for lipid rafts.

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