Improving the Phage-Displayed Oligopeptide:Magnetoelastic Particle Biosensors

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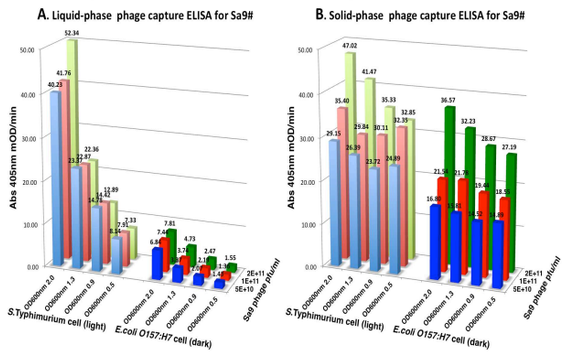
Rapid detection of biological threat agents (BTA) is essential to initiate a quick response for public safety. As demonstrated by several nationwide outbreaks of enteric pathogens in recent years, foodborne illness remains a serious public health concern in the United States. Therefore, a rapid and highly selective detection technology that can accurately detect BTA even in a complex biological environment is essential. Ideal detection technology should be fast (minutes rather than hours), accurate, have a long shelf life, inexpensive, and relatively easy to use without extensive training or elaborate equipment. Combinatorial chemistry of the phage display technology offers an ideal approach to develop specific peptide-based molecular probes that are stable and inexpensive for detection of BTA. The magnetoelastic (ME) particles are perfect biosensor platforms for phage-displayed peptide probes because they are inexpensive but highly sensitive to mass perturbance resulting from probe:analyte interactions. The ME biosensors using phage-displayed oligopeptides as probes have the advantage over other technologies because they are fast, cost effective, and require minimal sample manipulations. In our continuing effort to advance the phage-ME biosensors, we made several improvements for this technology. Firstly, we implemented a stringent negative selection procedure that minimized isolation of molecular probes with high cross reactivity. Secondly, we improved the classic biopanning and ELISA procedures to more accurately assess the interaction between molecular probes and whole bacterial cell analytes. Using these two improved approaches, we isolated highly selective phage-displayed oligopeptide probes for detection of *Salmonella enterica* serovar Typhimurium. Finally, we developed a genetic scheme to immobilize phage-displayed peptide probes on ME biosensors as a uniform monolayer to enhance capturing of analytes.

Traditional biopanning is performed with a library of random phage displayed peptides or antibodies against an analyte of interest. Although this procedure has been successful, it frequently yields probes with a high level of cross reactivity against other closely related analytes. In order to minimize isolation of probes with low selectivity, we implemented a negative selection step to remove probes with high cross reactivity. The negative selection is performed with analytes that are closely related but different from the target of interest to eliminate those probes that bind to common epitopes found among the analytes. Once nonspecific probes are removed, then the resulting clean library is panned against the analyte of interest to isolate probes that are specific to the target molecule. These simple steps result in isolation of highly selective phage-displayed peptide probes. We also determined that liquid phase biopanning and ELISA were superior to the solid phase assays when using live bacterial cells as the target. There are several problems that need to be addressed when live bacterial cells are used as the target for biopanning and ELISA on solid surface. First, the cells need to attach to the plastic surface. We discovered a large variation even among closely related bacteria for their ability to attach to the solid surface and this affected isolation of specific probes. Second, the most common method to attach bacterial cells to a solid surface involves drying the cells on the plastic. Unfortunately, both the attachment and drying alter cell surface properties that in turn affect isolation of probes with high selectivity. Although much more labor intensive, liquid biopanning, in which the cells and the probes are freely suspended, eliminates the problems associated with the solid phase biopanning. Third, we determined that the same problems associated with solid phase biopanning affected the ELISA assays for assessing the binding affinity of the probes to the targets. Thus, we developed a liquid phase ELISA in which all of the reactions are performed in suspension. Using these improved approaches for biopanning, we isolated highly selective phage displayed oligopeptide probes for detection of *S.* Typhimurium. The phage display library was negatively selected against 20 other serovars of *Salmonellae*. The resulting phage library was then selected for binding to *S.* Typhimurium by both solid and liquid phase biopanning. We present evidence that liquid biopanning yielded probes with higher selectivity than the solid phase biopanning. We also compared the traditional solid phase ELISA to the new liquid phase ELISA (Figure 1). We determined that liquid phase ELISA yielded results with less background and clearly distinguished selective probes from less selective probes.



Presently, phage probe are bound to the ME platforms in random and non-uniform fashion. Unfortunately, this may mask the oligopeptide probes and interfere with their function of capturing specific analytes. Thus, we developed a molecular genetic approach to improve the binding of phage probes on ME to optimize presentation of the oligopeptide probes for capturing specific analytes. Our approach is based on the construction of affinity-tagged derivatives of gpIX fusion protein of Ff class of filamentous phages to tether the phage probes as a monolayer on polymer coated ME particle. This approach can be utilized to 1) mutate *gIX* of any Ff class of filamentous phages and 2) achieve 100% affinity tagging of gpIX on any phage probe. As a proof of concept, we successfully tagged 100% of our phage particles with StrepTag II and demonstrated the efficacy of our genetic approach to improve the phage probe immobilization on the ME biosensor platforms.

In summary, we present several improvements to the current phage-ME biosensor technology to facilitate rapid detection of biological threat agents.