PHAGE AS A RECOGNITION ELEMENT FOR BIOSENSORS

Viswaprakash Nanduri[1], Alexandre M. Samoylov[1], Bryan A.Chin[3] Vitaly J.Vodyanoy[1] and Valery A. Petrenko[2]
[1] Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University.
[2] Department of Pathobiology, College of Veterinary Medicine, Auburn University.
[3] Department of Mechanical Engineering, Auburn University

As a part of a project for environmental monitoring of bio threat agents, this work was done to determine if phage could be used as a recognition molecule on a sensor. β-galactosidase from *E.coli* was used as a model threat agent. Binding of the selected phage to β -galactosidase was characterized by enzyme linked immuno sorbent assay (ELISA) in which the phage were immobilized on the plastic surface of the ELISA wells and reacted with their antigens in solution phase. Binding was measured by the ELISA signal, expressed as a slope of increase of optical density at 409 nm. As can be seen from figure 1, the binding of the phage (1) is dose dependant and the control (phage 2) showed negligible binding, even with increased concentrations of β-galactosidase.
The phage (1) was immobilized onto gold sensors by physical adsorption and the binding of the phage to β-galactosidase was studied. A cleaned, gold surface of a thickness shear mode (TSM) sensor was exposed to a phage suspension at a concentration of $3.2x10^{11}$ virions/mL for 1 hour and followed by washing with Dulbecco's phosphate buffered saline. After that, the sensors were placed in a wet chamber at $4\ ^0$ C for 24 hours. The sensor was exposed to a series of β-galactosidase solutions and the signal was recorded using a TM 400 Maxtek thickness monitor with a frequency resolution of 0.05Hz at 5 MHz. From the results obtained from 22 independent experiments it can be clearly seen that signals are dose dependent in a range of 0.003 to 210 nM. Figure 2, exemplifies dose-dependent binding of β-galactosidase to the phage (1) immobilized to the acoustic wave sensor. It was observed that apparent K_D of the complex was 2.8 nM ± 1.1 (S.D.) in TSM quartz sensor. The affinity valences of 2.3 ± 0.8 (S.D.) were estimated.

The results show that phage can be used a recognition element and can replace antibodies in biosensors. The study also shows the possibility of replacing antibodies with phage as recognition element.

Acknowledgement
This work was supported by Army Grant DAAD19-01-10454 (V.P), DARPA MDA 972-00-1-0011(V.V) and Detection and Food Safety Center of Auburn University Peak of Excellence.

Fig 1

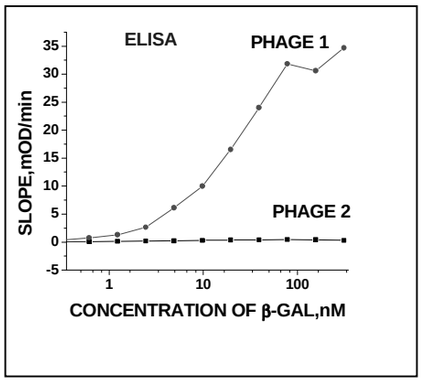

Fig 2