

Application of SPR to Bacteriology: Endotoxin/Protein Interaction Studies

Endotoxin (commonly referred to as lipopolysaccharide in bacteriology) is associated with the outer membrane of Gram-negative bacterial pathogens such as *Escherichia coli*, *Salmonella*, *Shigella*, and *Pseudomonas* [1-2]. The interaction between endotoxin and bacterial cell surface is schematically depicted in Figure 1. Endotoxin elicits a series of pleiotropic effects on cells or organisms and is therefore harmful to most mammals. The most serious consequence of Gram-negative bacterial infection is caused by the circulation of endotoxin and is termed as 'endotoxic shock' [2]. An endotoxin molecule is less potent when it is membrane-bound. Thus pathological effects arise when endotoxin molecules are released from multiplying cells. It is known that endotoxin and its complexes with certain proteins in plasma can interact with the CD-14 protein, which is a type of receptors on monocytes, macrophages and endothelial cells. This application note describes a novel application of SPR to the detection of endotoxin *via* its interaction with the CD-14 protein.

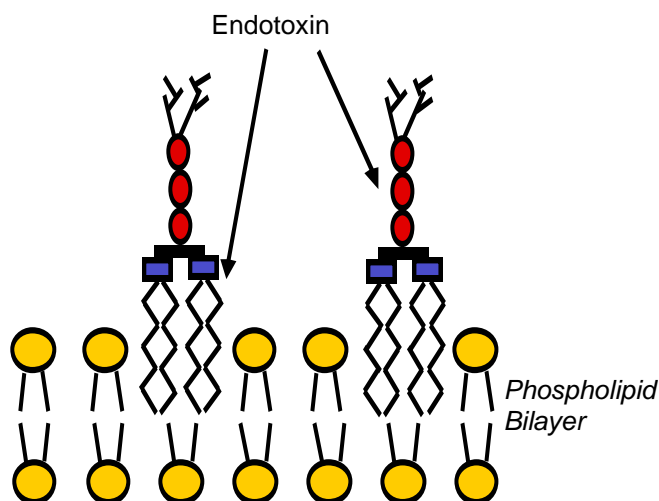


FIG. 1 The interaction of endotoxin with phospholipid bilayer of the outer membrane of Gram-negative bacterium. The yellow circles represent the lipid head groups and red ovals are the cores of endotoxin, and the branches are antigens. Endotoxin molecules are embedded in the bilayer via insertion of their lipid A chains into the membrane. For clarity, endotoxin and phospholipid molecules are not drawn to scale.

Before the experiment, a Au sensor chip was coated with a carboxymethylated dextran film. The formation of the carboxymethylated dextran is straightforward and follows a protocol described in literature [3]. Pyrogen-free water was used as the carrier (running) solution. Upon activation of the carboxyl groups on the carboxymethylated dextran with NHS/EDC, CD14 protein solution was injected into the SPR flow cell. CD14 can be effectively attached to the activated dextran film. Upon deactivation of the resultant surface with 1 M ethanolamine, solutions containing different amounts of endotoxin were injected and the binding curves were recorded. Figure 2 is an overlay of a series of binding curves.

Using a molecular weight of 10,000 Daltons for endotoxin, a K_D value of 37 nM was deduced from the simulation and suggests that endotoxin binds strongly to CD-14 protein. Furthermore, the association (k_a) and dissociation (k_d) constants were determined to be $8.0 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and 0.029 s^{-1} , respectively. Thomas and Surolia measured the kinetics of the interaction between endotoxin and polymyxin B (a cationic decapeptide) and determined the K_D value to be about 157 nM [4]. Comparable k_a and k_d values were also reported [4]. Thus, endotoxin appears to bind to CD14 almost as strongly as the peptide antibiotics. Moreover, an endotoxin concentration as low as 5 ng/mL can be easily detected. From this note, it is evident that SPR is a suitable technique for monitoring binding of endotoxins to proteins, antibiotics, and other species.

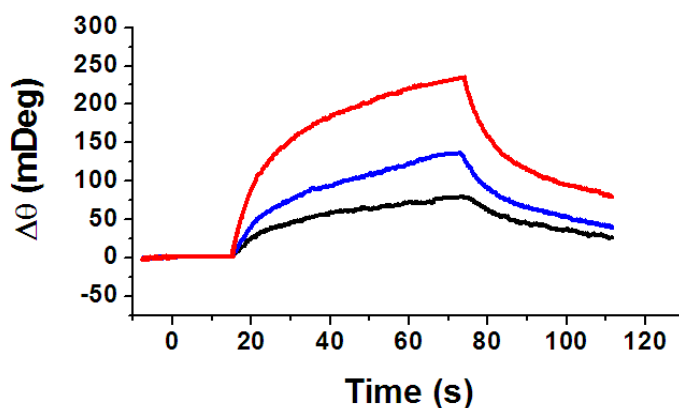


FIG. 2 Binding curves between pre-immobilized CD-14 protein and different concentrations of endotoxin: 20 ng/mL (red), 10 ng/mL (blue) and 5 ng/mL (black). A flow rate of 50 μ L/min was used.

Acknowledgement: We thank Jim Powers for his technical help, discussion, and the provision of the CD-14 protein and endotoxin samples.

References

- [1]. Y.L. Tzeng, A. Datta, V.K. Kolli, R.W. Carlson and D.S. Stephens, *J. Bacteriol.* 184, 2379-88(2002).
- [2]. C.R.H. Raetz, *Annu. Rev. Biochem.* 59, 129-170(1990).
- [3]. X. Yao, X. Li, F. Toledo, C. Zurita-Lopez, M. Gutova, J. Momand and F. Zhou, *Anal. Biochem.* 354, 220-228(2006).
- [4]. C.J. Thomas and A. Surolia *FEBS Lett.* 445, 420-424(1999).

Biosensing Instrument, Inc.

1 West Elliot Rd., Suite 111, Tempe, AZ 85284
 Tel: (480) 491-2777, Fax: (866) 897-8741
 info2bi@BiosensingUSA.com
 www.BiosensingUSA.com