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.

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.

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References