Binding Kinetics Analysis with SPR: Interaction between Bovine Serum Albumin (BSA) and Anti-BSA

In this application note, the use of a Biosensing Instrument(BI) flow injection SPR for real-time monitoring of the association and dissociation of anti-bovine serum albumin (anti-BSA) antibody with surface-confined BSA molecules is demonstrated. In restriction digestion, BSA has been used to stabilize enzymes during DNA digestion. It is also widely used as a biomolecule to block active sites on surfaces. Formation of the anti-BSA/BSA immune complex is relevant to studies of the receptor site of the red blood cells [1]. By injecting anti-BSA samples of different concentrations onto BSA-modified gold sensor chips and fitting the resultant sensorgrams with the BI Kinetic Analysis Program, the anti-BSA/BSA association and dissociation rate constants can be deduced.

BSA was first immobilized onto a carboxylated PEG/PEG [2] mixed monolayer in both SPR flow channels. Figure 1 shows the activation of the carboxyl groups on the PEG film with 0.1 M N-hydrosuccinimide (NHS) and 0.4 M N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide (EDC), followed by the adsorption of BSA by injecting 39 μM BSA dissolved in the same running buffer. To deactivate the NHS ester groups on the surface that have not reacted with BSA, 1 M ethanolamine (EA) solution dissolved in water was injected.

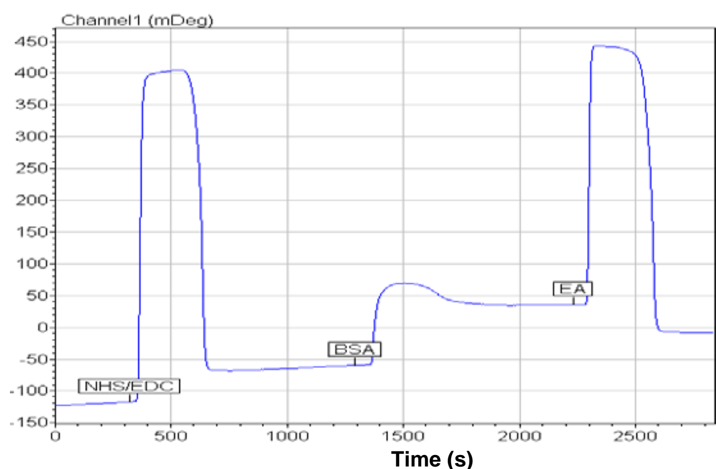

FIG.1 A SPR sensorgram showing the activation of the PEG film with NHS/EDC, the attachment of BSA via amine coupling, and the deactivation of the unreacted surface sites with ethanolamine. The flow rate was 20 μL/min and the running buffer is a phosphate buffered saline solution. The experimental apparatus is the same as that used for Application Note 105. The symbol before each peak indicates the solution injected into the SPR flow cell.

Next, a series of anti-BSA solutions were injected into the sample channel (channel 1 in this example), while the running buffer was flowing through the other SPR channel (or channel 2), which served as the reference channel. Figure 2 depicts the reference-subtracted sensorgram corresponding to the injections of the anti-BSA solutions and the regeneration of the sensor chip with 0.01 M HCl between two consecutive sample injections. Notice that the injection of an anti-BSA solution resulted in a rapid rise in the baseline, indicating that the association reaction between BSA and anti-BSA is rather facile. This is in contrast with the dissociation reaction, which showed a slow decay. Such a slow decay suggests that the binding of anti-BSA to BSA is quite strong. The third injection peak in Figure 2 corresponds to the injection of 80 nM anti-BSA. Notice that the injection of 0.01 M HCl did not completely regenerate the surface (as reflected by the baseline that is higher than that before the anti-BSA solution injection). Therefore, another surface regeneration step was carried out, which successfully changed the SPR signal back much closer to the original baseline. Notice, 150 nM anti-BSA was injected again, which produced an injection peak (fourth peak in Figure 2) that is comparable to the third injection peak.

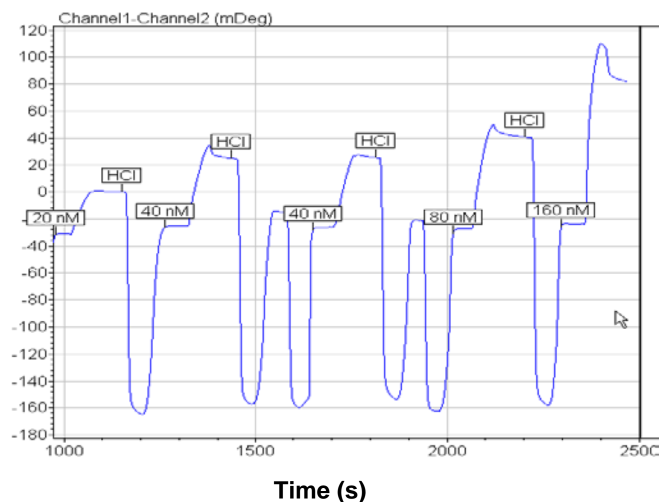

FIG.2 A SPR sensorgram showing the injections of 20, 40, 80, and 160 nM anti-BSA solutions (*cf.* the labels before the injection peaks). The negative peaks arise from the injection of the 0.01 M HCl regeneration solution that causes a large refractive index change in the bulk solution and the desorption of the anti-BSA from the complex. The flow rate was 80 μL/min and a loop of 100 μL was used.

The binding profiles in the above SPR sensorgram were cropped with the BI Data Analysis Program. These profiles can be conveniently selected from any desirable portions or durations of the sensorgram. The selected profiles can be readily imported into the Kinetic Analysis Program. Figure 3 displays the overlaid SPR binding profiles (black curves) at 4 different concentrations, together with the fit for the first order kinetics (1:1 type of interaction). As can be seen, the fit is excellent and yields an associated rate constant k_a of $8.2 \times 10^5\ M^{-1}s^{-1}$ and a dissociate rate constant k_b of $6.4 \times 10^{-4}\ s^{-1}$. Since the dissociation constant K_D is the ratio of k_d over k_a, we deduced the binding constant to be 7.9 nM. All these values are in reasonable agreements with other studies [3]. After these experiments, we injected anti-IgG into the reference channel and did not observe any binding between anti-IgG and BSA (data not shown). The lack of binding here indicates that the interaction between BSA and anti-BSA is highly specific.

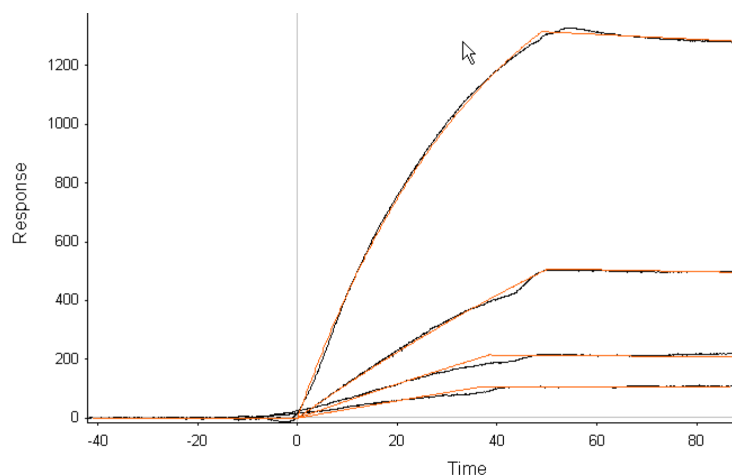

FIG.3 Experimental (black curves) and fitted (red curves) SPR binding profiles at different anti-BSA concentrations. The units for the SPR signals have been automatically converted from mDeg to response units.

References

[1] Varga, L., Thiry, E., Fust, G. *Immunology*, **1988**, 64, 381-384.

[2] Sigal, G. B., Bamdad, C., Barberis, A., Strominger, J., Whitesides, G. M. *Anal. Chem*. **1995**, 68, 490-497.

[3] www.colby.edu/chemistry/PChem/Lab/SI

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