Application Note # 147

Analyzing Dissociation Kinetics of IgG from Protein A Using MP-SPR and PureKinetics™

IgG dissociation kinetics from immobilized Protein A was studied using real-time Multi-Parametric Surface Plasmon Resonance (MP-SPR). Various dissociation buffers were tested to determine the most efficient solution. A unique feature, PureKinetics™, allows differentiation of real binding from interfering bulk signal artifacts, providing a pure binding signal. The method can clear out even extremely large bulk signals, such as high-ionic strength dissociation buffers. In this study, the most efficient dissociation from Protein A was achieved with buffers of pH below 4.0.

Introduction

Surface Plasmon Resonance (SPR) is a well-established method for molecule-molecule interaction studies. The SPR is a popular real-time and label-free method measuring association and dissociation kinetic constants in addition to the affinity constant. Multi-Parametric Surface Plasmon Resonance (MP-SPR) is based on the SPR principle, but it utilizes a unique optical setup which allows to solve challenges related to the buffer composition changes during SPR measurements.

In non-labelled optical methods, changes in buffer composition frequently cause a shift of the peak minimum angle value due to a change in refractive index of the buffer (called bulk effect or solvent effect). The usual method to counteract this is to subtract a reference channel response from the measurement channel response. However, the bulk effect is often not exactly the same in the different measurement channels because ligands might prevent the liquid flow near the surface. Sometimes suitable reference surface is hard to find, especially in the case of interactions to a membrane protein or interactions to coatings. Therefore, better methods than reference channel correction to compensate for bulk artifacts are needed.

Many small molecule weight drugs have poor solubility in water-based liquids, and solubility enhancers such as dimethyl-sulfoxide (DMSO) are needed to obtain suitable solubility. As the organic solubility enhancers create a large bulk signal in SPR, they also create huge challenges to assays in terms of a protein stability and function. Traditionally the bulk effect caused by DMSO is corrected using an empirical calibration curve requiring multiple injections [Frostell-Karlsson et al. 2000].

The unique optical setup of the MP-SPR instruments enables measurement of multiple optical parameters simultaneously and in real-time. Cross-correlation of the parameters allows simple in line characterization of the interfering bulk signal using the PureKinetics™ feature.

When is the PureKinetics™ useful?

- with Kinetic measurements of small molecules
- with Interaction measurements on lipid bilayers
- when there are Material interactions with biomolecules
- in case of Solvent interactions
- when using Crude samples (serum, growth medium, lysate, seawater)

Materials and methods

Protein A was immobilized covalently on a planar carboxymethyldextran (CMD-P) sensor surface using amino-coupling chemistry. A standard immobilization protocol was used: the surface was cleaned with a mixture of 2 M NaCl and 0.01 M NaOH before activation with N-hydroxysuccinimide (NHS, 0.05 M) and N(-3-dimethylamino-propyl)-N-ethylcarbodiimide hydrochloride (EDC, 0.2 M). The optimal pH for protein immobilization was screened on non-activated CMD (pre-concentration test) before immobilizing the Protein A. 10 mM sodium acetate pH=4.6 was found to give the best conditions for immobilization and was thus used in the experiments. 1 M ethanolamine pH=8.0 was used for surface deactivation. A regeneration buffer (glycine/HCL, pH=2.0) was used to remove bound IgG between experiments.

IgG dissociation from the Protein A was studied using 15 different buffers and results of five of these are shown here:

1. PBS + 1 M NaCl
2. 0.1 M sodium phosphate + 1 M NaCl, pH=3.0
3. 0.1 M sodium phosphate + 1 M NaCl, pH=1.5
4. 0.1 M glycine, pH=4.5 + 0.02 M ethyleneglycol
5. 0.2 M sodium carbonate pH=11.5

A 96-well plate automated MP-SPR Navi™ 220-L NAALI instrument was used for the experiments. Measurements were performed in angular scan measurement mode at 21˚C, using a flow rate of 20 µL/min. Dissociation rates were calculated using TraceDrawer™ for MP-SPR Navi™.

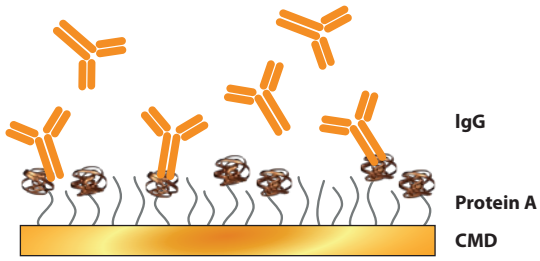

Figure 1. Schematic view of Immunoglobulin G dissociation from Protein A.

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Results and discussion

The binding level of Protein A was initially 31 mdeg (17 ng/cm^2). The immobilization level was lower due to the lysine fragments that were used for the biotinylation of Protein A causing less groups being available for NHS/EDC coupling to the dextran surface. However, the level of immobilization was sufficient for the dissociation experiments. The saturated binding level signal of IgG was approximately 110 mdeg. No reference channel was needed for the experiment as the bulk effect was measured in line.

MP-SPR method using the PureKinetics™ feature was capable of measuring the dissociation rate of IgG from Protein A in different dissociation buffers even when the dissociation buffers had significantly higher refractive index than the running buffer. Figure 2 shows a standard SPR sensogram (A) and the PureKinetics™ sensogram (B) during dissociation measurement using buffer 1. The unique MP-SPR PureKinetics™ sensogram shows the binding without the bulk effect and allows IgG dissociation rate (k_d) to be determined from the sensogram. This determination is not possible from the standard SPR sensogram (A) as the dissociation is covered by the bulk signal.

The dissociation rate was clearly slower with the buffer 1 and 5 compared to the other three dissociation buffers (Figure 3). The magnitude (effectiveness) of dissociated IgG was determined and showed a lot of variation depending the buffer used. Dissociation was the most effective with buffer 3 and least effective with buffer 1 (Figure 4).

Conclusions

MP-SPR provides high quality kinetic data even with buffer compositions greatly varying within the same experiment. Effective internal referencing was made possible by the PureKinetics™ feature, which provides more reliable results from a simpler assay than traditional SPR. PureKinetics™ is extremely beneficial in pre-clinical drug development, in interaction studies, and when an appropriate reference surface is not available such as measurements with lipid bilayers or bio-coatings. MP-SPR is the only instrument providing the PureKinetics™ feature.

References:

Frostell-Karlsson et al. (2000). J. Med. Chem 43, 1986-1992.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 420A ILVES, 220A NAALI or 210A VASA

Sensor surfaces: CMD-P

Software: MP-SPR Navi™ Control, DataViewer, and TraceDrawer™ for MP-SPR Navi™.

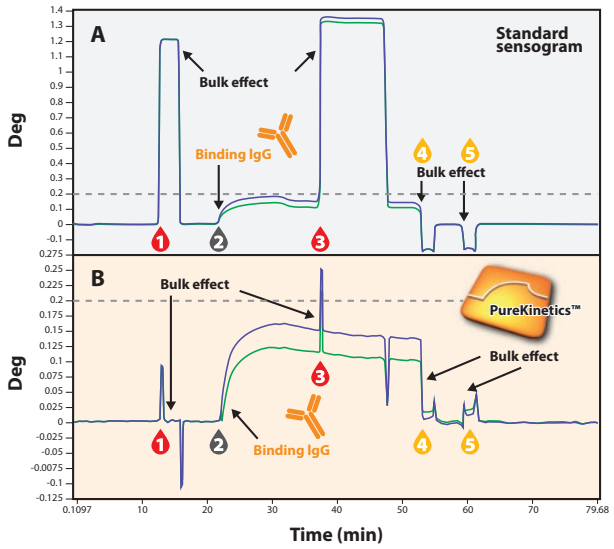

Figure 2. (A) the standard SPR sensogram and (B) the PureKinetics™ sensogram of the same experiment. Measurements were done in parallel (blue and green). The standard SPR sensogram shows large responses due to the refractive index differences (bulk effect) between running buffer and dissociation or regeneration solutions. The PureKinetics™ sensogram shows the true binding response without bulk artifacts. Using the PureKinetics™ feature, the IgG dissociation in changing buffers could be quantified.

Events in the sensogram: 1. dissociation buffer PBS + 1 M NaCl, 2. IgG 33 µg/mL, 3. dissociation buffer PBS + 1 M NaCl 4. and 5. regeneration solution glycine/HCL pH=2.0

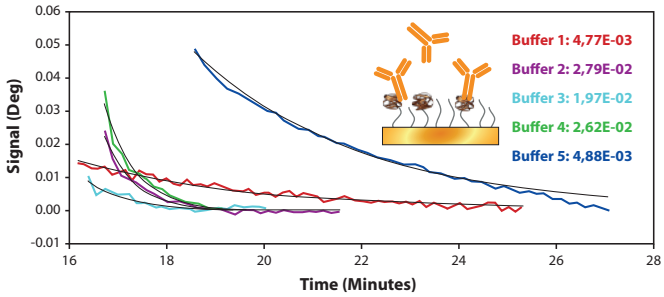

Figure 3. The IgG dissociation rate constants (k_d) in different buffers. Colored curves represent the measured dissociation data, and the black curves the fit to the data.

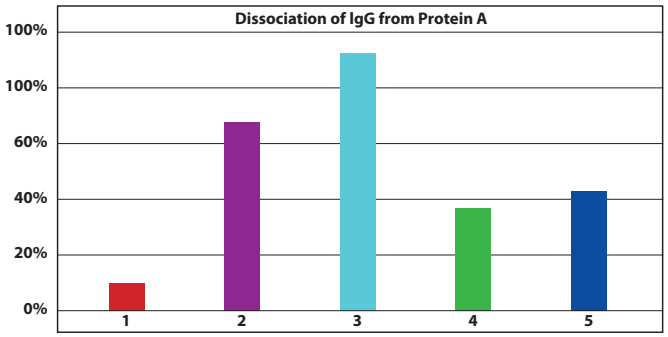

Figure 4. Percentage of the IgG dissociation in different test buffers. A higher percentage means more dissociation. 1. PBS + 1 M NaCl, 2. 0.1 M sodium phosphate + 1 M NaCl, pH=3.0, 3. 0.1 M sodium phosphate + 1 M NaCl, pH=1.5, 4. 0.1 M glycine, pH=4.5 + 0.02 M ethyleneglycol and 5. 0.2 M sodium carbonate pH=11.5

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