Binding Kinetics of Therapeutic Monoclonal Antibodies to the CD16a Fc Receptor using OpenSPR™

Summary

- Fc receptor CD16a binding to monoclonal antibodies were analyzed using OpenSPR™
- Kinetic analysis was used to determine the on rate, off rate, and affinity constant of the CD16a-IgG interaction, a critical assay in development and production of monoclonal antibody therapeutics
- OpenSPR provides an affordable benchtop solution for this and many other important applications

Overview

CD16a (FcγRIIIA) is an Fc receptor protein found on the surface of certain immune cells. CD16a binds to the Fc region of immunoglobulin G (IgG). CD16a-IgG binding initiates antibody-dependent cellular cytotoxicity (ADCC), an important effector mechanism of the immune system. CD16a assays are essential for the development and production of therapeutic monoclonal antibody (mAbs).
In this application note, OpenSPR™ is used to successfully determine the binding kinetics and affinity of human CD16a interacting with human IgG (Fc region). OpenSPR allows users to easily and accurately measure the binding kinetics of numerous receptor-ligand systems.

Materials and Equipment

- OpenSPR™ Instrument
- TraceDrawer Kinetic Analysis Software
- Sensor Chip: OpenSPR Streptavidin Sensor
- Buffer: filtered and degassed 20 mM HEPES, 150 mM NaCl, pH 7.4, 1% BSA, 0.005% Tween 20 (HBS-EP)
- Regeneration: 10 mM glycine-HCl in diH_2O
- Ligand: Biotinylated Human CD16a
- Analyte: Human IgG (Fc Fragment)

Procedure

1. Following the start-up procedure found in the OpenSPR manual, set up the OpenSPR instrument and software.
2. Condition the surface by performing 2-3 injections of 10 mM glycine-HCl at 150 µL/min.
3. Set the pump speed to 20 µl/min. Inject 150 µl of 1/5 µg/ml CD16a.
4. Condition the surface by performing one injection of 10 mM glycine-HCl at 150 µL/min.
5. Change the flow rate to 20 µL/min. Inject 150 µl of running buffer to be used as a reference for kinetic analysis. Wait 10 minutes. Prepare three 200 µl samples of IgG diluted in the running buffer at the following concentrations: 111 nM, 333 nM, and 1000 nM.

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6. Prepare three 150 µl samples of IgG diluted in the running buffer at the following concentrations: 111nM, 333nM, and 1000nM.
7. Inject the lowest concentration of IgG, and after 10 minutes have passed inject the next highest concentration. Repeat until all concentrations have been injected.
8. Evaluate the experimental data with TraceDrawer. Subtract the buffer reference curve from the binding curves and fit with a 1:1 kinetic model.

Results and Discussion

The sensograms are shown below in *Figure 1*. A 1:1 binding model was used to fit the data. The kinetics constants were determined to be (standard errors are given in brackets):

Off Rate (k_{off})	5.40e-3 s-1 (+/- 6.95e-7)
On Rate (k_{on})	4.27e4 M-1*s-1 (+/- 8.11e1)
Equilibrium Constant (K_D)	126 nM (+/- 0.26)

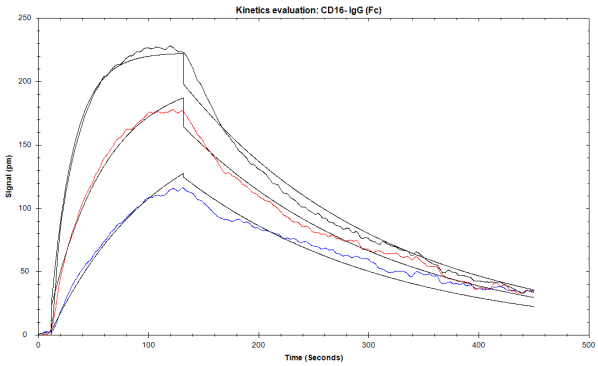

Figure 1. The interaction between CD16a and IgG measured with the OpenSPR instrument. Kinetic analysis was performed using a 1:1 model in TraceDrawer. IgG concentrations (from the bottom to the top): 111nM, 333nM, 1000nM

Conclusions and Summary

This study demonstrates how OpenSPR can easily be used to determine the binding kinetics of therapeutic monoclonal antibodies and the CD16a receptor. OpenSPR's affordable price point and benchtop size makes it an important tool for the development of new monoclonal antibody therapeutics and biosimilars.

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