



Kinetic & Affinity Characterization – Polyclonal Antibodies

OBJECTIVE

To determine the kinetics of the interaction between two different polyclonal antibodies and their respective peptide and protein antigen.

CONCLUSIONS

- A method has been developed that allows for real time, label-free measurement of polyclonal antibody interactions to peptide as well as protein antigens, using the Attana A100[®] C-Fast system.
- By using this method, kinetic information about the interactions, such as association (k_{on}) and dissociation (k_{off}) rate constants can be derived and the affinity of the interaction calculated.
- The method can be used to follow purification of polyclonal antibodies, compare immunizations, monitor titer changes and antibody maturation.
- The assay is time saving and cost effective as it screens polyclonal antibodies against an immobilized antigen.

BACKGROUND

The primary goal of antibody production is to obtain high titer, high affinity antiserum for use in experimentation, diagnostic tests or therapy. Detection and monitoring of the purification of these polyclonal antibodies can be time consuming and troublesome. This Application Example demonstrates that the Attana A100 C-Fast system can be used to rapidly characterize polyclonal antibodies raised against peptides and protein antigens, respectively. Polyclonal antibodies are by nature a mixture of antibodies, with their own respective kinetic characteristics and most probably also with differences in antigen epitope specificities. Due to this heterogeneity, the rate constants derived and the calculated affinity should be regarded as a mean of the different subpopulations.

ATTANA A100 C-FAST BIOSENSOR

The Attana A100 C-Fast biosensor utilizes the Quartz Crystal Microbalance (QCM) technique for real time, label-free measurements of molecular interactions. When molecules are added to, or removed from the sensor surface, the change in the resonance frequency corresponds to the change in mass on the surface. By immobilizing a target molecule to the sensor surface, and flowing an interacting molecule over the surface, the interaction can be studied in real time. The real-time information can provide kinetic, affinity and specificity data on the interaction.

METHOD

Kinetic determination of the interaction between a polyclonal antibody and a biotinylated peptide antigen: The biotinylated peptide (1 ng/ml) was immobilized on a streptavidin coated Attana Biotin Chip using PBST (10 mM Phosphate Buffer Saline containing 0.005% Tween[®] 20) as running buffer at a flow rate of 25 μ l/min. The purified polyclonal antibody, recognizing the biotinylated peptide antigen, was diluted to 10, 5, 2.5, 1.25 μ g/

ml and subsequently injected over the surface. Between each antibody injection, the surface was regenerated by one injection of glycine-HCl pH 2.5, for a contact time of 30 s.

Kinetic determination of the interaction between a polyclonal antibody and a 15 kDa protein antigen: An Attana Carboxyl Sensor Chip was equilibrated in the A100 C-Fast instrument and 50 μ l of the protein antigen, 0.1 μ g/ml in HAc pH 5.0, was immobilized on the surface by means of EDC/sulfo-NHS-mediated amine coupling, using 10 mM HEPES as running buffer at 23°C and 25 μ l/min. The interaction experiments were conducted in PBST by injecting the polyclonal antibody at 5, 2.5, 1.25, 0.625 and 0.3125 μ g/ml. The surface was regenerated by 100 mM H₃PO₄, followed by 200mM NaOH, for 45 s and 30 s, respectively.

Buffer was injected before and after each concentration series and was used as a reference blank.

Data was collected by Attester[™] v3.0 and subsequently processed in Attester[™] Evaluation v3.0. To achieve kinetic data, Attester Evaluation and Clamp XP^{*} were used. For the rate constant calculations, 150 kDa was used as the molecular weight of the polyclonal antibodies.

RESULTS

The compiled data obtained for the interactions between the two different polyclonal antibody sera, one raised against a peptide and the other against a protein, and their antigens, are shown in Fig. 1 and Fig. 2 respectively. The highest concentration of the polyclonal antibodies used, 10 and 5 μ g/ml, resulted in a frequency response of 12 and 20 Hz, respectively. To control that the interactions were unaffected by mass transport limitations, several flow rates were tested and 25 μ l/min was found to be appropriate for the interaction studies. After compilation of the



Kinetic & Affinity Characterization – Polyclonal Antibodies

data sets, a global kinetic analysis of the binding curves, using the Clamp XP software, resulted in a calculated K_D of 7.2 nM for the polyclonal antibody targeted against a peptide antigen (Fig. 1) and 0.3 nM for the polyclonal antibody targeted against a protein antigen (Fig. 2).

As mentioned, the heterogenic nature of the polyclonal antibody sample, will most probably lead to a mixed display of different affinities. This will also have implications for the possibility to fit simple kinetic interaction models to the data. Graph A in Fig. 1, shows a rather high off-rate in the first part of the dissociation phase, indicative of subpopulations forming relatively unstable complexes with the antigen. Thereafter, the off-rate stabilizes, representing the subpopulations forming relatively stable complexes with the antigen, and data obtained for this part of the dissociation phase show a better agreement with the 1:1 interaction model.

Moreover, when using an assay design with immobilized antigens on the sensor surface, for the characterization of bivalent, or multivalent antibodies, great care has to be taken to measure affinity rather than avidity. We have previously

shown that, using the Attana Carboxyl or Biotin Sensor Chips, the surface antigen density can be titrated with great precision to avoid avidity effects caused by high surface densities. This was done for both of the antigens before initiation of the experiments presented here.

Attana Materials Used	Item Code
Attana Carboxyl Sensor Chip	3616-3033 (pack of 3)
	3616-3103 (pack of 10)
Attana Biotin Sensor Chip	3613-3033 (pack of 3)
	3613-3103 (pack of 10)
Amine Coupling Kit	3501-3001
Streptavidin	3502-3001
C-Fast: 3.1	3420-3001
Attester™: 3.1	3410-3001
Attester™ Evaluation: 3.1	3430-3001

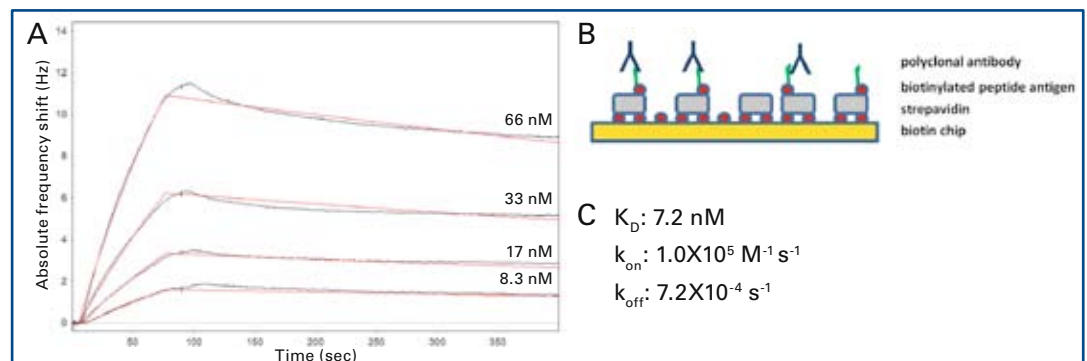


Figure 1: Binding of a polyclonal antibody to a biotinylated peptide antigen. (A) Binding curves (black) and fit of the binding to a simple 1:1 binding model (red). (B) Schematic illustration of the assay set up. (C) Derived rate constants and K_D calculated from k_{off}/k_{on} .

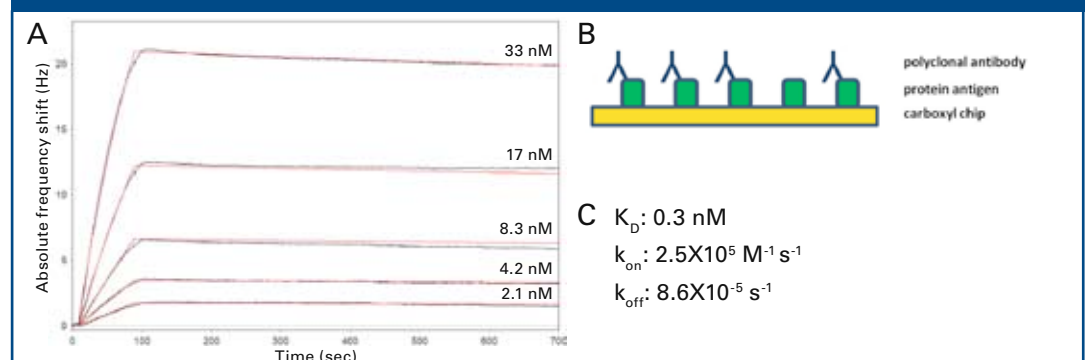


Figure 2: Binding of a polyclonal antibody to an amine coupled protein antigen. (A) Binding curves (black) and fit of the binding to a simple 1:1 binding model (red). (B) Schematic illustration of the assay set up. (C) Derived rate constants and K_D calculated from k_{off}/k_{on} .

Attana AB

Björnnäsv. 21. SE-113 47 Stockholm, Sweden
Telephone: +46 8 410 200 00, E-mail: info@attana.com
www.attana.com