Analytical Biochemistry 518 (2017) 110–125

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Bivalent kinetic binding model to surface plasmon resonance studies of antigen-antibody displacement reactions

Dagmar Gelinsky-Wersing [a], Wolfram Wersing [b, *, 1], Wolfgang Pompe [a]

[a] Institute for Materials Science and Max Bergmann Centre of Biomaterials, Technische Universität Dresden, 01062 Dresden, Germany
[b] Siemens AG, Corporate Technology, 81739 München, Germany

ARTICLE INFO

Article history:
Received 23 May 2016
Received in revised form
9 November 2016
Accepted 17 November 2016
Available online 22 November 2016

Keywords:
Small molecule sensor
SPR detection
Protein interaction
Ligand binding
Kinetic rate constants
Kinetic simulation

ABSTRACT

Molecular and functional analysis of small molecule binding to protein can provoke insights into cellular signaling and regulatory systems as well as facilitate pharmaceutical drug discovery. In label free small molecule detection the displacement assay format can be applied. This is beneficial because displacement of high molecular weight receptors is detected instead of low molecular weight ligand as in classical binding analysis. Thus, detection limit is potentially lowered.

Using the influenza haemagglutinin (HA) peptide binding to mono or bivalent anti-haemagglutinin peptide antibody displacement assay formats could be established. The exact time resolved analysis of binding and dissolution of ligand HA and anti-Haemagglutinin peptide antibody was achieved with surface plasmon resonance (SPR) spectroscopy.

Mathematical models could be developed from kinetic equations of ligand binding to mono or bivalent antibodies. With this, an accurate simulation of the SPR results was reached. The simulation plot had to be exactly adjusted to the SPR results to determine all kinetic rate constants defining ligand and receptor binding kinetics. Large variations in receptor concentration gave almost identical rate constants in binding. It became obvious that rebinding is in any case not necessary to understand the binding kinetics of our model system HA/anti-HA. Maximum decline of SPR response could be used to determine ligand concentrations in analyte.

1. Introduction

For many biological phenomena the binding of small molecules (molar mass below 800 g/mole) to receptors is of immense interest. Small molecules are for example many controlling molecules such as pheromones or second messengers; also pesticides and most of the drugs are composed of small molecules [1]. The analysis of the binding of small molecules to proteins is important for the understanding of biological control systems and in pharmaceutical research, e.g. in high-throughput screening [2] for medical active substances. Simple strategies to analyze the binding of small molecules will be increasingly important for fragment based drug discovery (FBDD) a quite new method to build up step by step new active substances from small molecules. Although, each single molecule binds only weakly to a specific biological target the tailored combination of several small molecules can result in a strong affinity to that target [3].

There are several assay formats available to study the binding of small molecules to receptors or to measure the concentration of small molecules. In most cases a particular molecular property such as fluorescence or radioactivity of the interacting molecules is required [4,5]. However, labeling of molecules in solution requires elaborate cleaning processes and furthermore, there can be a risk that complex formation between ligands could occur. Therefore, label free detection principles are preferred which usually monitor changes in mass or refractive index close to the sensor surface using acoustic resonance or surface plasmon resonance detection, respectively. Unfortunately, the mass sensitivity limit of these detection principles is even under optimal conditions only around 100 g/mole and thus, the resonance signal change due to ligand binding of small molecules with molecular weights (MW) below 500 g/mole is too low to be detected [6]. This is even more critical if immobilized receptors of high molar mass are used.

In the case ligands bound to a receptor are easily accessible, a second receptor of higher molarity is introduced which can bind to

* Corresponding author. Wagnerfeldweg 10, 83346 Bergen, Germany.
E-mail address: wolfram.wersing@t-online.de (W. Wersing).
[1] Retired.

http://dx.doi.org/10.1016/j.ab.2016.11.012
0003-2697/

the ligand. Thus these so called sandwich assays enable the amplification of the detector signal [7].

When the binding behavior of low molecular weight ligands is of interest it is useful to couple the ligand to the transducer surface and to detect the binding of receptors which are usually of higher molarity [2].

Some quantitative methods are based on competition assays similar to classical immunoassays. In these cases exact amounts of ligands and receptors are mixed together in a way that the ligand concentration can be easily calculated from the concentration of receptors binding on the transducer surface. Often these kinds of assays are used to find an inhibitor for a specific ligand-receptor binding and are therefore named inhibition in solution assay (ISA) [8]. Sometimes the binding of a mixture of a free ligand and a modified competitor ligand on the receptor surface is analyzed (surface competition assay, SCA) [9].

A further label free detection principle, the displacement or replacement method [10], is also very promising for the detection of small molecules. Similar as with ISA the binding of receptors to immobilized ligands competes with the binding to free ligands. However, this does not happen at the same time but successively, i.e. the association processes of receptors to immobilized ligands (association phase) and the processes of free ligands to replace bound receptors (displacement phase) take place at different time intervals. This detection method is based on the quasi stationary measurement of the ligand receptor complexes at the end of both the association and the displacement phase. Obviously, quasi-stationary solutions of the reaction equations exist for both phases.

In this paper we refer to the displacement method to study the kinetics of association and displacement processes in the model antigene-antibody system haemagglutinin (HA) peptide/haemagglutinin specific antibody (HA antibody). This model system allows the study of two alternative interaction modes by considering low- and high-affinity antibodies. In order to obtain more information than simply the binding constants the binding of the macromolecules was measured with high precision in real time using a SPR spectrometer. Changes in the refractive index, close to the sensor surface, are monitored using SPR. Stenberg and co-workers [11] have shown that for proteins changes in the refractive index are proportional to the accumulation of mass.

In order to carefully separate association and displacement processes, a further phase, the dissociation phase, is inserted between the association and the displacement phase. In this phase the receptor solution is washed out by a buffer solution and the loss of bound antibodies is monitored as a function of time. Altogether, during these three phases (association, dissociation, and displacement phase) the concentrations of ligand-receptor complexes have been precisely monitored with high temporal resolution.

A full characterization of the interaction kinetics demands a larger set of reaction constants which describe the association, the dissociation and the displacement of the reaction partners. To measure these rate constants, time-dependent solutions of the reaction kinetics are needed. These have been obtained by integrating the system of kinetic differential equations numerically using MATLAB code. By adapting the calculated time dependent concentrations with the measured ones over all three phases it was possible to determine the rate constants with good precision. This has been achieved with a binding model for monovalent antibodies (monovalent model, MM) and also with a model for bivalent antibodies (bivalent model, BM). It will be shown that problems with monovalent binding models such as rebinding and multivalent binding effects can be overcome with the aid of the introduced bivalent binding model.

2. Materials and methods

2.1. Instrumentation and reagents

The SPR-spectrometer BIOSUPLAR 3 by Analytical μ-Systems (Sinzing, Germany) including measurement software was used for the interaction analysis. In our system we used two spots in separated flow channels on the sensor surface. These two channels of the flow cell obtained from Analytical μ-Systems (Sinzing, Germany) can be fed with solutions separately therefore one channel can be used as reference channel. The flow rates were typical 60 μl per minute. The SPR angles of the spots were collected every 0.1 s and stored as data files readable by different data analysis programs.

2.1.1. Antibodies and antigens

Monoclonal anti-HA antibodies (clone HA-7) synthesized from mice, and the antigene Influenza haemagglutinin peptide (HA) have been obtained from Sigma-Aldrich Chemical Co. (Deisenhofen, Germany). High affinity antibodies F_{ab} fragments (clone BMG-3F10) rats IgG_1 have been delivered by Roche Diagnostics GmbH (Mannheim, Germany).

2.1.2. Buffer solutions

Phosphate buffered saline (PBS), pH 7.4 has been obtained from Sigma-Aldrich Chemical Co. (Deisenhofen, Germany). 10 mM acidic phosphate buffer, pH 4.5 has been prepared by dissolving 275.6 mg $NaH_2PO_4H_2O$, 0,53 mg $Na_2HPO_42H_2O$, and 2.24 g KCl in 200 mL ddH_2O followed by adjustment to pH 4.5 using 1 M NaOH.

2.2. Design and performing the binding assay

To study the displacement of HA specific antibodies by free HA peptide a HA peptide affinity matrix was deposited onto the transducer surface that enable the specific binding of HA specific antibodies.

For this purpose a self-assembling monolayer (SAM) of disulphide-PEG[2]-carboxylic acid (4,7,10,13,16,19,22,25,32,35,38,41,44,47,50,53-Hexadecaoxa-28,29-dithiahexapentacontanedioic acid; Sigma-Aldrich Chemical Co. (Deisenhofen, Germany)) was bound to the gold surface and the carboxylic acid was chemically activated using a buffer solution containing 0.2 M N-ethyl-N′-dimethylaminopropyl carbodiimide (EDC) from Sigma-Aldrich Chemical Co. (Deisenhofen, Germany)) and 0.05 M N-hydroxysuccinimide (NHS) from Sigma-Aldrich Chemical Co. (Deisenhofen, Germany)). The activated carboxylic acid can be nucleophile attacked by the primary amino group of the HA peptide thereby forming a covalent amid binding.

2.2.1. Self-assembling monolayer (SAM)

To prepare the self-assembling monolayer (SAM) a SPR gold slide from Analytical μ-Systems (Sinzing, Germany) was cleaned by immersing it three times for 20 s in un-denatured ethanol (purity 99.5%, Carl Roth GmbH, Karlsruhe) in an ultrasonic bath followed by immediate immersion in 1 mM solution of disulphide-PEG-carboxylic acid (S_2-PEG-COOH) in un-denatured ethanol. The glass vessel used was sealed with parafilm (Sigma-Aldrich Chemical Co., Deisenhofen, Germany) and incubated for at least 12 h at room temperature.

The *cleaning of the gold surface* is of immense importance for preparing homogenous SAM. In order not to hinder the kinetics of the SAM organization both gold surface and solution must be completely free from contaminations [12]. However, due to the

[2] PEG: Polyethylene glycol.