Colloids and Surfaces B: Biointerfaces 117 (2014) 425–431

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Accounting for unintended binding events in the analysis of quartz crystal microbalance kinetic data

Gabriella T. Heller[a], Theodore J. Zwang[a], Elizabeth A. Sarapata[b], Michael A. Haber[a], Matthew H. Sazinsky[a], Ami E. Radunskaya[b], Malkiat S. Johal[a,*]

[a] Chemistry Department, Pomona College, 645 North College Avenue, Claremont, CA 91711, United States
[b] Mathematics Department, Pomona College, 610 North College Avenue, Claremont, CA 91711, United States

ARTICLE INFO

Article history:
Received 7 December 2013
Received in revised form 13 February 2014
Accepted 28 February 2014
Available online 12 March 2014

Keywords:
QCM-D
Unintended interactions
Nonspecific interactions
Kinetics
Dissociation constant

ABSTRACT

Previous methods for analyzing protein–ligand binding events using the quartz crystal microbalance with dissipation monitoring (QCM-D) fail to account for unintended binding that inevitably occurs during surface measurements and obscure kinetic information. In this article, we present a system of differential equations that accounts for both reversible and irreversible unintended interactions. This model is tested on three protein–ligand systems, each of which has different features, to establish the feasibility of using the QCM-D for protein binding analysis. Based on this analysis, we were able to obtain kinetic information for the intended interaction that is consistent with those obtained in literature via bulk-phase methods. In the appendix, we include a method for decoupling these from the intended binding events and extracting relevant affinity information.

1. Introduction

The quartz crystal microbalance with dissipation monitoring (QCM-D) should be well suited for analyzing protein–ligand binding kinetics, due to its high sensitivity and rapid rate of data collection. Despite this, it is difficult to accurately measure affinity constants found using previously described methods with the QCM-D [1,2]. A major problem is the use of functional protein surfaces that can never be thought of simply as an array of only the intended binding sites, even though they are often treated as such. Proteins tend to orient randomly when they adsorb to a surface, which can compromise the availability of their receptor sites for ligands. It is also common that a given protein attached to a surface will not cover the entire exposed area. Inhomogeneities in surface adsorption create variation in the surface density of intended ligand binding sites between surfaces and create regions outside of these intended sites that can bind ligands, both of which can dramatically obscure analysis of kinetics on proteins surfaces.

Methods currently exist for determining binding rate constants using a QCM-D, but they generally lack comparisons to literature values or rigorous theoretical studies that would establish their validity [3]. None of these methods take into account the effects of unintended binding events that are always present to some degree in surface measurements. Furthermore, most methods assume a linear relationship between rate constants and bulk phase ligand concentration. In this article we present a new method for analyzing ligand-binding data from a QCM-D that accounts for unintended mass bound to the sensor and does not assume a linear relationship between rate constants and bulk phase ligand concentration. To address such problems, it is important to understand how the morphologies of protein surfaces relate to well-established kinetic models.

Single step protein–ligand complex formation, both on surfaces and in solution, can be described most simply by Eq. (1).

$$[\text{Protein}] + [\text{Ligand}] \rightleftarrows [\text{Complex}] \quad (1)$$

The forward rate constant, k_{on}, relates protein and ligand concentration to the amount of complex that is formed over time. The reverse rate constant, k_{off}, relates the amount of complex to the rate at which it dissociates back to the free protein and ligand. These two constants are important characteristics of the system and they can be used to predict both the observed rate of complex formation and the equilibrium state of the system. It is common

* Corresponding author. Tel.: +1 9096074253.
E-mail address: malkiat.johal@pomona.edu (M.S. Johal).

http://dx.doi.org/10.1016/j.colsurfb.2014.02.044
0927-7765/

to determine the rate constants by fitting experimental data to the following equation:

$$\frac{d[\text{Complex}]}{dt} = k_{on}[\text{Protein}][\text{Ligand}] - k_{off}[\text{Complex}] \quad (2)$$

We refine this model and test it on three systems: (1) the binding hemin to human serum albumin (HSA), (2) the binding of Fe(III) 2,5-dihydroxybenzoic acid complex ($Fe(2,5\text{-}DHBA)_3$) to neutrophil gelatinase-associated lipocalin tagged with glutathione S-transferase (NGAL-GST), (3) the binding of caffeine to bovine serum albumin (BSA). All three of these systems have been well characterized in the literature and behave differently in the QCM-D with varying affinities and reversibilities. This is especially important in this context as it tests the limits of our model and drastically different, but well understood systems. Multiple binding partners with well-characterized affinities were used to strengthen the validity of this model.

Characteristics of the QCM-D binding data for these three systems that are inconsistent with Eq. (2) are (1) a non-constant deposition rate in the association phase, (2) a non-zero mass near the steady state of the rinse phase, (3) a non-linear dependence on ligand concentration and (4) a non-constant ligand concentration for runs lasting short periods of time. Our model accounts for these factors and demonstrates the feasibility of using QCM-D to extract kinetic information and accurately determine affinity constants (K_d) for protein–ligand complexes.

2. Materials and methods

2.1. QCM-D setup

Real-time frequency and dissipation data were collected using QCM-D (E4, Q-Sense, Gothenburg, Sweden) using four different concentrations. Frequency data were obtained at the fifth overtone. The QCM-D sensor consisted of an AT-cut piezoelectric quartz crystal disk coated with a gold electrode on the underside and an active surface layer of gold (100 nm thick).

QCM-D liquid handling fluidics were decontaminated by flowing a solution of 2 vol.% Hellmanex solution (Hellma GmbH & Co., Müllheim, Germany) for 30 min, then mQ (<18 MΩ cm resistivity) water for 30 min and finally air for 5 min.

All QCM-D crystals were optically polished with a root-mean-square roughness of less than 3 nm. Crystals were decontaminated by UV-ozonation for 10 min, treated with a 1:1:5 (vol.) solution of ammonium hydroxide, hydrogen peroxide and water at 75 °C for 5 min, submerged in water and rinsed with ethanol.

To immobilize the protein, clean crystals were placed in a 10 mM solution of mercaptoundecanoic acid in methanol overnight, rinsed with methanol and dried. Crystals were then submerged in a solution of 5 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide (EDC/NHS) in mQ water for 2 h at 4 °C, rinsed with buffer and placed in the solution containing the corresponding protein sample (HSA = 2 mg/mL, 50 mM glutathione (GSH), BSA = 2 mg/mL) for 2 h at 4 °C. Then, the crystals were rinsed with mQ water and placed in a solution of 50 mM ethanolamine for 2 h at 4 °C [4]. The GSH-functionalized crystals were placed in a solution of NGAL-GST for 2 h at 4 °C. All crystals were rinsed with mQ water and dried with N_2 gas and then mounted in a liquid flow cell (40 μL) and operated at 4.95 MHz.

A stable baseline was obtained by flushing the QCM-D flow cell with phosphate buffered saline (PBS) for 1 h. Once this baseline was obtained, various concentrations of ligand were passed through the flow cells, followed by a PBS rinse. Due to the four-flow cell setup of the E4 QCM-D model, a single experiment consisted of monitoring the binding of ligand to four identically prepared protein-functionalized surfaces. Four unique concentrations of ligand were used for each experiment. Flow cell temperature was fixed at 25.00 ± 0.02 °C, and a peristaltic pump (Ismatec ISM935C, Wertheim, Germany) was used to flow solution through the cell at a constant rate of 300 μL/min. Details of QCM-D principles can be found elsewhere [5].

2.2. System one: HSA–hemin

Human serum albumin (Sigma–Aldrich, Saint Louis, MO) and hemin (Strem Chemicals, Newburyport, MA) were used as received. PBS, pH = 7.4 was used as buffer. Hemin solutions were prepared by first dissolving the solid in 2 vol.% 1 M NaOH, then diluting with PBS. The pH of these solutions was fixed to 7.4.

2.3. System two: NGAL-GST-Fe(2,5-DHBA)$_3$

NGAL expression and purification was followed according to Bundgaard et al. [6]. Protein was expressed in BL21 Star (DE3) *E. coli* cells with the pSJS 1240 plasmid for rare codons instead of XL1-Blue. Growths were done in Terrific Broth media with 100 mg/L amp and 70 mg/L spec. Induction lasted 4 h. DHBA (Matheson Co., Inc., Albuquerque, NM) was mixed in a 3:1 ratio with iron ammonium sulfate ($Fe(2,5\text{-}DHBA)_3$). Tris buffer pH = 7.4 was used.

2.4. System three: BSA–caffeine

Bovine serum albumin and caffeine (Sigma–Aldrich, Saint Louis, MO) were used as received. PBS, pH = 7.4 was used as buffer. Caffeine was first dissolved in 2% methanol and diluted with PBS.

2.5. Data fitting

Fitting was performed using Matlab's fminsearch optimization routine with ODE45 as the numerical solver (Version 7.5.0.338) solver. Code is available upon request.

3. Results and discussion

Due to randomness associated with orientation and surface packing, the number of functional surface-bound proteins varies by an unknown quantity between each created surface. In an attempt to minimize this complication, much work has focused on the use of surfaces that will attach proteins at inactive regions and force them to orient in a desirable way. This allows for greater control over the number and concentration of protein receptor sites, but the morphologies of these surfaces may still vary so they cannot be thought of simply as an array of receptors at constant concentration.

This presents a significant problem with surface methods, which is the possibility that ligands in solution will bind to sites other than the intended receptor. This can confound solution phase measurements as well, but the inclusion of a surface onto which ligands can bind exacerbates the problem. Ligands interacting with the surface at unintended sites can also be modeled by Eq. (2), if protein concentration is replaced by another term describing the available sites for this secondary interaction. For these measurements, the raw data can be described by Eq. (3) as the amalgamation of all binding events:

$$\frac{d[\text{Complex}_{\text{total}}]}{dt} = k_{on}[\text{Free Binding Site}][\text{Ligand}] - k_{-off}[\text{Complex}_{\text{intended}}] + \sum (k_i[\text{Unintended Binding Site}_i][\text{Ligand}] - k_{-i}[\text{Complex}_i]) \quad (3)$$