Determining the Binding Kinetics of HIV-1 Nucleocapsid Protein to Six Densities of Oligonucleotide Using the ProteOn™ XPR36 Protein Interaction Array System

Andrew Stephen, Karen Worthy, Lakshman Bindu, and Robert Fisher, Protein Chemistry Laboratory, Advanced Technology Program, SAIC-Frederick, Inc., National Cancer Institute at Frederick, Frederick, MD 21702 USA

Introduction

The ProteOn XPR36 protein interaction array system is a surface plasmon resonance (SPR) biosensor that can measure the interaction of up to six ligands with up to six analytes, producing 36 kinetic profiles simultaneously (Nahshol et al. 2008). Multiple conditions can be tested in parallel; therefore, a comprehensive kinetic analysis of an analyte concentration series can be performed in one experiment. The ProteOn XPR36 system can be used for comprehensive analysis of biomolecular interactions such as protein-protein and protein–nucleic acid.

In this study, we used the ProteOn XPR36 system to analyze the binding kinetics of the HIV-1 nucleocapsid (NC) protein, with a short deoxynucleotide, $d(TG)_5$. We created a NeutrAvidin capture surface to bind the biotinylated $d(TG)_5$ DNA followed by a single analyte injection of six different concentrations of NC. The data obtained were fit to a 1:1 Langmuir binding model to evaluate which ligand densities are appropriate for detailed kinetic analysis. The results confirmed the complex interaction between these two molecules that was demonstrated in our previous work (Fisher et al. 2006).

Methods

Instrumentation and Sensor Chip

All experiments were performed using the ProteOn XPR36 protein interaction array system (Bio-Rad Laboratories, Inc.) and one ProteOn GLC sensor chip (Bio-Rad).

Immobilization of Biotinylated $d(TG)_5$

The GLC sensor chip was preconditioned with three injections of 50 mM NaOH in 1 M NaCl in the vertical direction and three injections in the horizontal direction, with a contact time of 30 sec and a flow rate of 10 µl/min. ProteOn PBS/Tween (0.005% Tween 20), pH 7.4, was used as the running buffer. All six ligand channels were activated by injection of the amine coupling reagents 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC, 100 mM) and N-hydroxysulfosuccinimide (sulfo-NHS, 25 mM) (from the Bio-Rad ProteOn amine coupling kit) for 300 sec at 30 µl/min in the horizontal direction. NeutrAvidin (200 µg/ml, Pierce Biotechnology, Inc.) in 10 mM ProteOn acetate buffer, (Bio-Rad) was injected in the horizontal direction for 300 sec over the activated channels. Excess reactive amine groups were deactivated with a 300 sec injection of 1 M ethanolamine HCl (Bio-Rad). Using this approach, approximately 6,000 response units (RU) of NeutrAvidin were amine-coupled to the GLC sensor chip. Next, 2-fold dilutions of biotinylated $d(TG)_5$ (biotin-TGTGTGTGTG, Integrated DNA Technologies) ranging from 100–3.12 nM were made in PBS/Tween and injected for 30 sec at 50 µl/min in the vertical direction. Any unbound ligand was removed by regenerating the surface with a 30 sec injection of 0.1% SDS followed by a 30 sec injection of 1 M NaCl.

Nucleocapsid Binding Kinetics

NC protein (a gift from Dr Rob Gorelick, SAIC-Frederick) was diluted to 300, 100, 33, 11, and 3.7 nM in 10 mM HEPES, 150 mM NaCl (pH 7.5), 5 mM β-mercaptoethanol, 100 µM TCEP, 0.005% Tween 20, and 1 µM $ZnCl_2$. The five NC protein concentrations and a buffer control were injected in the horizontal analyte channel with a contact time of 180 sec, dissociation time of 900 sec, and flow rate of 100 µl/min. The ligand channels were regenerated with a 30 sec injection of 0.1% SDS followed by a 30 sec injection of 1 M NaCl. All experiments were run at 25°C.

Sensorgram Analysis

The six sensorgrams from the $d(TG)_5$ ligand channels were x and y transformed, and nonspecific binding was referenced using the interspot reference capability. Sensorgrams were double-referenced by subtracting the buffer response. The six sensorgrams were fit globally to a 1:1 Langmuir binding model, and the refractive index value was kept constant. The kinetic parameters for the association (k_a), dissociation (k_d), and R_{max} were derived from the fitted curves.

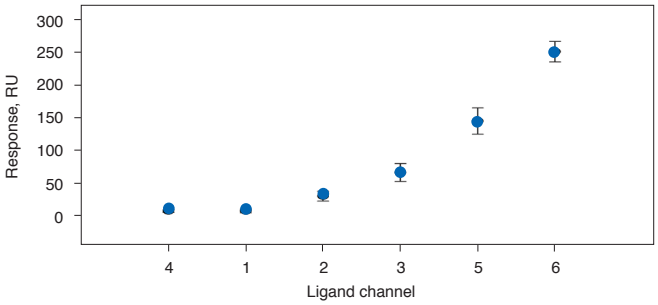

Fig. 1. Oligonucleotide densities. Six different concentrations of d(TG)$_5$, ranging from 3.12 to 100 mM, were injected onto the six channels of a NeutrAvidin-coated chip, creating six different density surfaces. Immobilization of the oligonucleotide on the chip was measured across all six interaction spots in a channel.

Table 1. Kinetic constants and ranking for NC binding to six different surface densities of d(TG)$_5$.

Channel	d(TG)$_5$ injected, nM	d(TG)$_5$ density, RU*	k_a, $M^{-1}sec^{-1}$	k_d, sec^{-1}	K_D, M	R_{max} RU	Chi^2
4	3.12	8.0 ± 1.9	4.31e^5	20e^{-3}	4.64e^{-9}	21.5	3.7
1	6.25	8.2 ± 2.9	3.65e^5	20e^{-3}	5.48e^{-8}	33.65	3.75
2	12.5	30.7 ± 7.3	2.94e^5	10e^{-3}	3.4e^{-8}	85.33	9.87
3	25	66.7 ± 13.6	2.07e5	8.91e–3	4.3e^{-8}	179.76	47.79
5	50	145.0 ± 20	1.78e5	4.64e–3	2.61e^{-8}	329.09	313.09
6	100	250.7 ± 15.6	1.71e5	2.4e–3	1.41e^{-8}	444.3	905.07

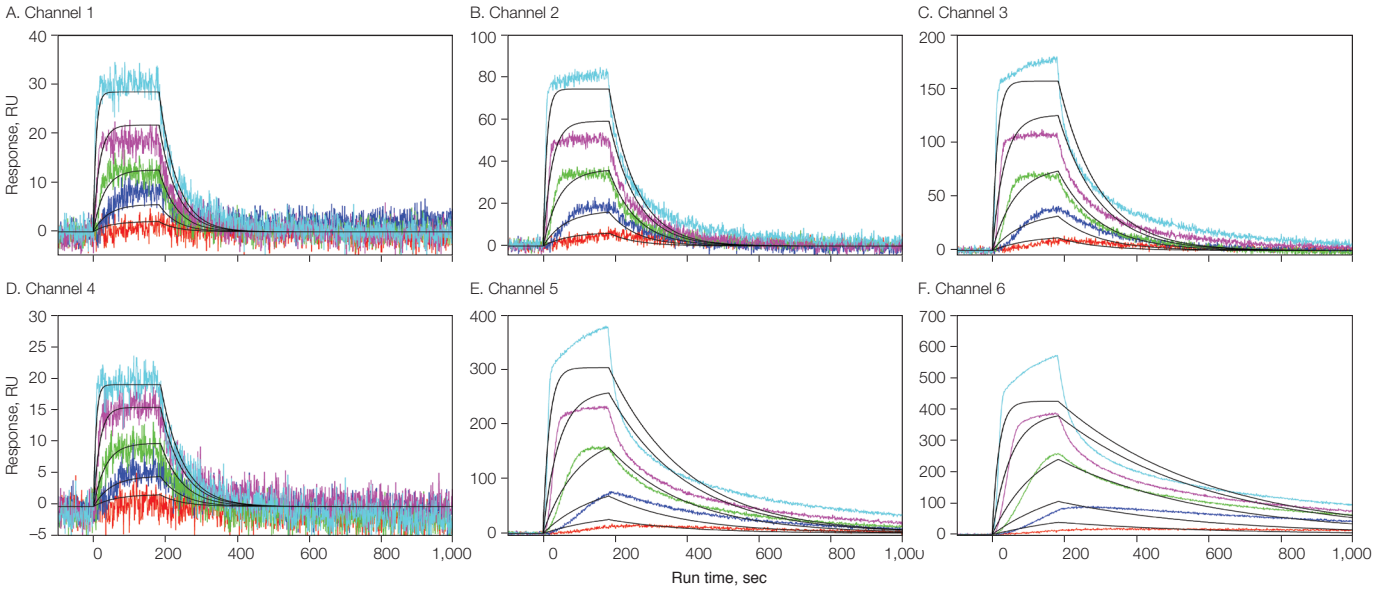

Fig. 2. Interaction kinetics of NC binding to different densities of d(TG)$_5$ are compared to the Langmuir 1:1 model. The black trace represents the global fit of the sensorgrams to the 1:1 interaction model. The interactions between six different d(TG)$_5$ ligand densities with five concentrations (—, 300; —, 100; —, 33; —,11; —, 3.7 nM) of NC were tested.

Results and Discussion

There was a linear relationship between the d(TG)$_5$ concentration injected and the amount of d(TG)$_5$ captured on each channel (Figure 1). The density of d(TG)$_5$ was determined by taking the mean value across all six interaction spots in the channel between 50–100 sec after injecting the oligonucleotide dilution series. The lowest concentration of oligonucleotide injected, 3.12 nM, gave a similar signal to channel 1.

The data highlighted in red in Table 1 (8.0 and 8.2 RU) represent sensorgrams that correlated well to the 1:1 Langmuir binding model as evaluated by chi^2 values of <5. At higher densities of d(TG)$_5$, the fit is not as tight; this is shown by the increase in the chi^2 values.

The sensorgrams from channels 1 and 4 show that the data obtained overlay the 1:1 Langmuir binding model very well (Figure 2). These sensorgrams correspond to the lowest immobilized levels of d(TG)$_5$, <10 RU. At these densities, the surface-bound d(TG)$_5$ molecules are sparse enough so that only one NC molecule can bind to each d(TG)$_5$ molecule. In channel 2, the data fit the 1:1 model at only the three lowest NC concentrations. When the oligonucleotide surface reaches higher densities (30, 67, 145, and 251 RU), the data do not fit well to the 1:1 Langmuir binding model because surface-bound d(TG)$_5$ molecules are close enough so that the NC is able to bind to more than one d(TG)$_5$ molecule at a time.

The equilibrium binding constant determined for the two channels with the lowest d(TG)$_5$ densities is approximately 46 nM, which is in the range of values measured in previous work (Fisher et al. 2006, Stephen and Fisher 2009).

Conclusions

The ProteOn XPR36 system performed well in measuring the binding kinetics of the NC protein to the $d(TG)_5$ oligonucleotide. We see a linear response between the amount of biotinylated oligonucleotide that was injected and the amount captured on the NeutrAvidin surfaces.

The NC protein interacted with different binding behaviors to the channels with differing $d(TG)_5$ densities as shown by changes to the closeness of the fit of the experimental data to the 1:1 Langmuir binding model. We were able to produce data on low-density surfaces of $d(TG)_5$ (≤10 RU) that fit well to the 1:1 Langmuir model. These data are consistent with our expectations and previous work (Fisher et al. 2006, Stephen and Fisher 2009). The unique array design of the ProteOn XPR36 system allows the investigator to rapidly evaluate the effect of different density capture surfaces on the binding kinetics of a protein target.

References

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