Generating and interpreting kinetic data in protein interaction studies

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Short abstract

By deconstructing protein interactions into association and dissociation phases, information is revealed on the molecular mechanisms underlying protein function that are not possible to attain from steady state analyses. Biacore™ X100 is used here to provide this information rapidly and with minimum user training.

Long abstract

The kinetics of the interaction of cystatin B, a protein inhibitor of cysteine proteinases involved in intracellular degradation of proteins, with papain was profiled using Biacore X100.

Specifically, interactions involving cystatin B in which tyrosine was replaced with alanine at position 97 in the C-terminal loop of the protein, as well as wildtype protein, were studied. The mutated protein bound papain with almost 30-fold reduced affinity compared with wildtype. Analysis of the interaction showed that the reduction in affinity was entirely due to an increase in the rate of dissociation, while the rate of association remained unchanged, possibly indicating a role for tyrosine 97 in consolidating the stability of the formed complex. The derived kinetic rate constants were in close agreement with data from a previous study (1) using more complex and time-consuming biochemical methods.

Kinetic analysis, by deconstructing affinity into association and dissociation rates, revealed a comprehensive, dynamic picture of the interaction between cystatin B and papain, providing clues about protein function unattainable from steady state assays.

Introduction

Protein interaction detection techniques that reveal affinity provide important information, but say nothing about the dynamic processes of association and dissociation, or the kinetics, of the interaction. Knowledge about these component phases may provide clues about how the details of interactions are important determining factors in the control of protein function.

Here, the interaction of cystatin B with papain was profiled using Biacore X100.
The data were used as the basis for a hypothesis on the functional role of the C-terminal loop structure of cystatin B.

Methods

Biacore assays are based on the phenomenon of surface plasmon resonance, a label-free and non-invasive technique. The analysis produces a plot of binding response in real time over the entire course of the interaction, providing information about on and off rates. A mutant of cystatin B was created, where the tyrosine residue at position 97 was replaced with alanine. Solutions of both wildtype and mutated cystatin B were injected over papain immobilized on the surface of a sensor chip.

Immobilization of papain: Papain was immobilized on the second of 2 flow cells using a standard amine coupling procedure with Na-acetate at pH 4.5 as a coupling buffer. 1 μg/ml papain was injected for 50 s resulting in approximately 50 RU of immobilized protein. Unmodified flow cell 1 was used as a reference surface.

Kinetic assay: Two series of native and mutated cystatin B, ranging in concentration from 1 to 40 nM, were injected using a single-cycle kinetics approach in which increasing concentrations of protein were sequentially injected over the chip surface with no intervening regeneration steps. After the final injection of cystatin B, the chip surface was regenerated using 20 mM NaOH for 30 s at a flow rate of 10 μl/min.

All interaction steps were carried out at 25°C.

Materials

Interacting partners in solution	Cystatin B variants, produced and purified as described (1)
Immobilized interacting partner	S-(methylthio) papain, with a methyl-thio group attached to Cys 25 in the active cleft
Instrument	Biacore X100 http://www.biacore.com/lifesciences/products/systems_overview/x100/system_information/index.html
Software	Biacore X100 Control Software; Biacore X100 Evaluation Software
Sensor chip	Sensor Chip CM5 http://www.biacore.com/lifesciences/products/Consumables/guide/cm5/index.html?1=
Amine Coupling Kit	GE Healthcare catalogue number, BR-1000-50 http://www.biacore.com/lifesciences/products/systems_overview/x100/related/index.html
Acetate buffer pH 4.5, 50 ml	GE Healthcare catalogue number, BR-1003-50 http://www.biacore.com/lifesciences/products/systems_overview/x100/related/index.html
HBS-EP+ buffer 10X, 4 x 50 ml	GE Healthcare catalogue number, BR-1008-26

http://www.biacore.com/lifesciences/products/systems_overview/x100/related/index.html

Plastic Vials Ø 11 mm	GE Healthcare catalogue number, BR-1002-87

http://www.biacore.com/lifesciences/products/systems_overview/x100/related/index.html

Rubber caps, type 2	GE Healthcare catalogue number, BR-1004-11

http://www.biacore.com/lifesciences/products/systems_overview/x100/related/index.html

Results

Sensorgrams showing the entire interaction profiles of native and mutated cystatin B with papain are shown in Figure 1. The instrument software converted data from the interactions into kinetic rate and affinity constants.

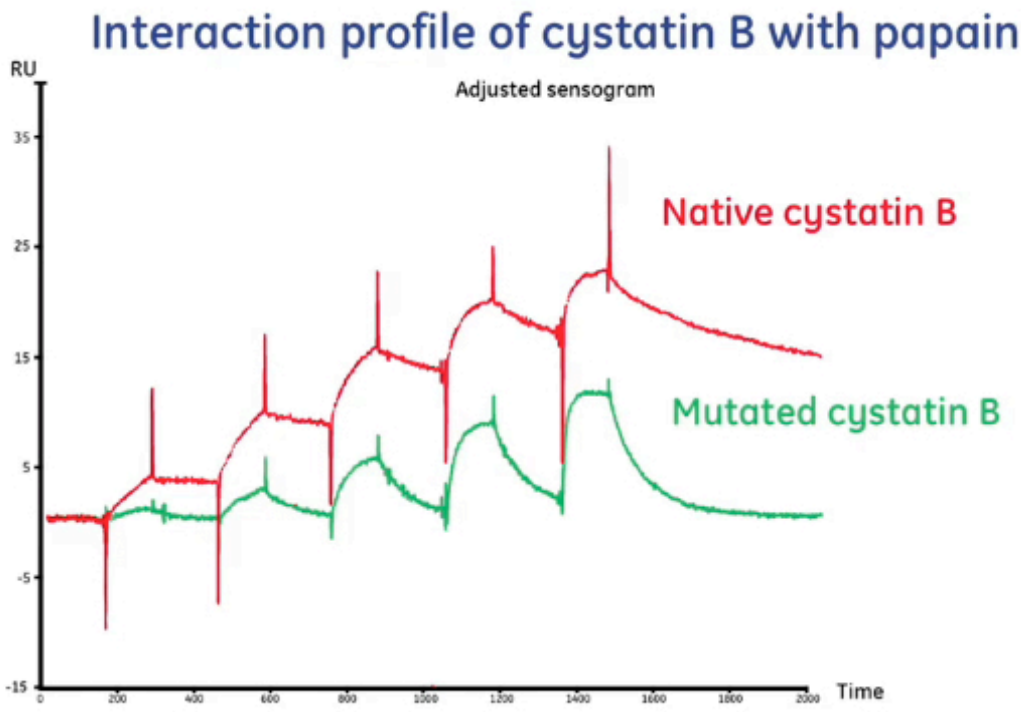

Figure 1. Profiles of interactions between cystatin B and papain. The peaks are the results of interactions involving progressive increases in the concentrations of cystatin B over the course of the experiment. No regeneration of the chip surface was necessary between injections.

The mutated protein bound papain with almost 30-fold reduced affinity. Kinetic analysis, however, revealed that this was caused entirely by an increase in the rate of dissociation, while the rate of association remained unchanged (Figure 2).

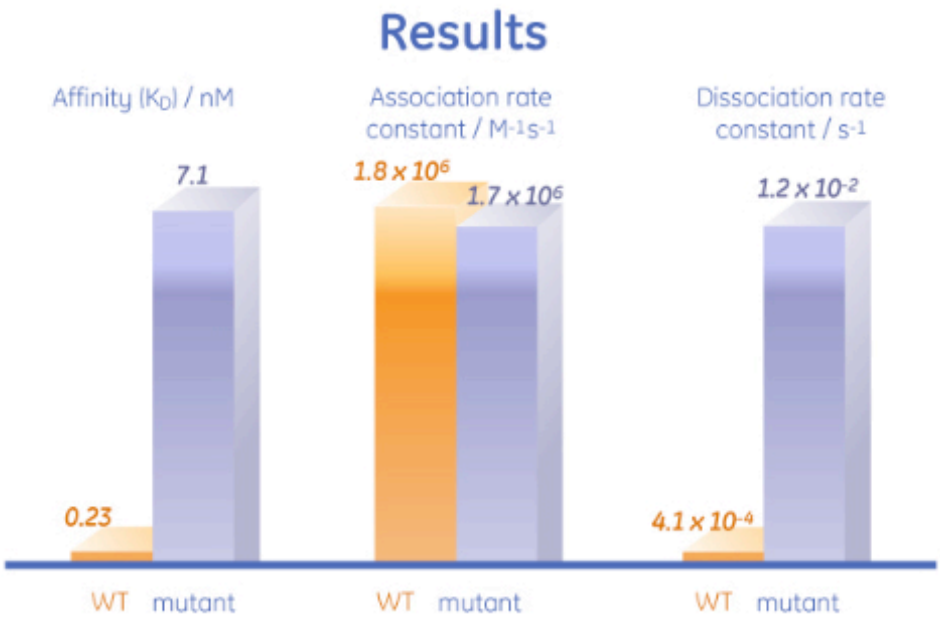

Figure 2. Affinities, association rate constants and dissociation rate constants of the interactions between wildtype (WT)/mutant cystatin B and papain.

Discussion

Kinetic analysis of the interaction between cystatin B and papain, from sample preparation to data analysis, took less than 4 hours. Replacement of tyrosine with alanine at position 97 in the C-terminal loop of cystatin B greatly reduced its affinity for papain. This was entirely due to an increase in the rate of dissociation. The derived kinetic rate constants were in close agreement with data from a previous study (1) using more complex and time-consuming biochemical methods.

Although steady state assays reveal a picture of an interaction at equilibrium, they reveal nothing about rates of association and dissociation, important indicators of recognition and complex stability. These issues can only be resolved through kinetic analysis, used here to identify a specific tyrosine residue in the C-terminal loop of cystatin B, and suggesting that it may be responsible for preserving the stability of complexes formed with papain by functioning as an anchor.

This example shows how kinetic analysis using Biacore X100, by revealing the whole dynamic picture of protein interactions, can help reveal how proteins function and their role in the behavior of the whole cell.

Reference

1. Pol, E. & Björk, I. Importance of the second binding loop and the C-terminal end of cystatin B (stefin B) for inhibition of cysteine proteinases. Biochemistry 38, 10159-10526 (1999).