Analysis of Multiple Protein-Protein Interactions Using the ProteOn™ XPR36 Protein Interaction Array System

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Introduction

Many biological systems require analysis of multiple protein-protein interactions to further the understanding of their function. One example of such an analysis is the investigation of the relative contributions of specific protein substructures and residues to the binding interface between TEM1 β-lactamase (TEM1) and the β-lactamase inhibitor protein (BLIP) (Figure 1) (Albeck and Schreiber 1999). TEM1 β-lactamase is one of over 200 different β-lactamases found in nature. These versatile enzymes are present in both gram-positive and gram-negative bacteria, and are the principal mechanism of resistance to antibiotics. BLIP is a secreted protein from the soil bacterium Streptomyces clavuligerus that inhibits a wide range of β-lactamases.

Mutated TEM1 residues were used to analyze the consequences of mutations on the binding energetics of the protein interface. Just as the change in free energy of binding between a mutant and wild-type protein indicates the relative energetic contribution of each residue to the total binding energy, changing multiple residues in concert permits the extent of cooperativity among residues to be examined. In this tech note we report on the kinetic analysis of the binding interactions of five TEM1 mutant proteins with BLIP as a model for constructing a high-resolution picture of the noncovalent interactions within the TEM1/BLIP binding interface. Through kinetic analysis, the relative change in free energy (ΔΔG) due to a mutation can be determined by comparing the affinities of binding with ($K_{A, mut}$) and without ($K_{A, wt}$) the mutation (Albeck and Schreiber 1999). Central to this analysis was the fast and accurate "one-shot kinetics" capability of the ProteOn XPR36 protein interaction array system (Bronner et al. 2006a). The ProteOn XPR36 surface plasmon resonance (SPR) optical biosensor incorporates a 6 x 6 multichannel module and an interaction array sensor chip for analysis of up to 36 protein interactions in a single, rapid experiment.

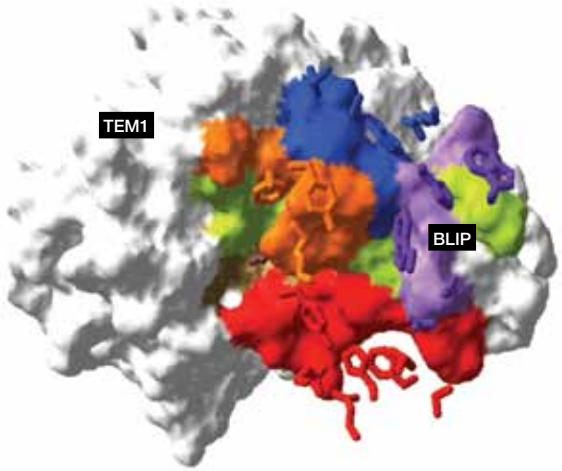

Fig. 1. Space-filling model of the binding interface between TEM1 and BLIP. The binding interface was resolved into five distinct modules (shown in color), with each module consisting of the noncovalent interactions of corresponding residues on each protein. See Reichmann et al. (2005) for details.

For a more complete description of the scope of the interactions measured, the methods used to derive binding energetics from kinetic constants, and a detailed analysis of the architecture of the TEM1/BLIP interface made possible by this experimental design, see Albeck and Schreiber (1999), Reichmann et al. (2005), and Bronner et al. (2006b).

Methods

Instrumentation, Samples, and Reagents

The experiment was performed using the ProteOn XPR36 protein interaction array system, with one ProteOn GLC sensor chip, at 25°C. The protein expression and purification procedures used to prepare TEM1 (29 kD) mutant protein and wild-type BLIP (17.5 kD) samples are described by Albeck and Schreiber (1999). The five TEM1 mutants used were R243A/S235A, R243A/S130A, S130A/S235A, K234A, and E104A. ProteOn phosphate buffered saline with 0.005% Tween 20, pH 7.4 (PBS/Tween) was used as running buffer.

Sensorgram Analysis

A set of 36 sensorgrams was generated during a single injection step of the six BLIP samples and grouped into six sets of six. Each sensorgram set was processed for baseline alignment and reference channel subtraction.

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Determination of Kinetic Rate Constants

Kinetic analysis was performed by globally fitting curves describing a simple 1:1 bimolecular reaction model to each set of six sensorgrams to obtain the association rate constant, k_a, and dissociation rate constant, k_d, from which the equilibrium dissociation constant, K_D, was calculated from the relationship $K_D = k_d/k_a$.

Protein-Protein Interaction Analysis

For immobilization of the five TEM1 mutant proteins, five of the six ligand channel surfaces were activated by injection of the amine coupling reagents 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC, 100 mM) and *N*-hydroxy-sulfosuccinimide (sulfo-NHS, 25 mM) (components of the ProteOn amine coupling kit).

A 200 µl sample of each of the five TEM1 mutant proteins (2 µM, prepared in sodium acetate buffer, pH 4.0) and a buffer sample were injected at a flow rate of 30 µl/min in one injection cycle. To deactivate remaining carboxyl groups in the five channels, 1 M ethanolamine HCl, pH 8.5 (ProteOn amine coupling kit) was then injected. A sample of running buffer was included in each step for injection into the sixth channel, which was used as a reference channel.

Next, a serial dilution of BLIP samples was prepared at 600, 300, 150, 75, 37.5, and 18.75 nM in PBS/Tween, pH 7.4. Samples (150 µl) of each concentration were injected into the six analyte channels orthogonal to the TEM1 ligand channels at a flow rate of 100 µl/min, generating the 36-element interaction array. The binding kinetics for the interactions of each of the five TEM1 mutant proteins with BLIP were then rapidly and accurately obtained in "one shot" on a single chip (Bronner et al. 2006a).

Results and Discussion

Of the six sets of sensorgrams obtained, five corresponded to the interaction of the six BLIP concentrations with one of the TEM1 mutant proteins (Figure 2), and one set to the reference channel (not shown). The kinetic constants for each TEM1 mutant protein with BLIP are shown in Table 1. Note that the affinities of the interactions varied significantly, indicating the relative contributions of the mutated residues to the binding interface.

Table 1. One-shot kinetic values for the interaction of BLIP and TEM1 mutants.

TEM1 Mutant	k_a ($M^{-1}sec^{-1}$)	k_d (sec^{-1})	K_D (nM)
R243A/S235A	1.51×10^4	5.09×10^{-4}	33.8
R243A/S130A	1.27×10^4	1.24×10^{-3}	97.6
S130A/S235A	3.10×10^4	9.33×10^{-4}	30.1
K234A	2.01×10^4	8.50×10^{-4}	42.3
E104A	1.70×10^5	7.40×10^{-3}	43.5

While a detailed interpretation of these results requires knowledge of the placement of each residue within the interface (as may be obtained from structural analysis by crystallography), and also supplemental kinetic data obtained from analysis of the interactions between the wild-type protein forms (not shown) and other mutants for proper evaluation of the free energy changes induced by each mutant protein, a few conclusions may be reached from the limited data presented here.

Each of the TEM1 mutations analyzed slightly weakens the TEM1-BLIP interface. The lower affinity with BLIP is indicated by the reduced association and enhanced dissociation rate constants of the TEM1 mutant proteins relative to TEM1 wild type. For example, removal of positively charged residues from TEM1, as represented by TEM1 K234A (lysine to alanine), results in a significant reduction of the association rate constant due to lessened electrostatic attraction with a corresponding aspartic acid residue on BLIP during the association phase. However, removal of a negative charge from TEM1 causes little change in the association rate constant, as represented by TEM1 E104A (glutamic acid to alanine).

The contribution of each mutated residue to the stabilization of the complex can be seen by the effect on the dissociation rate constants. The observed change in dissociation rate constants depends on the alteration of the specific interactions that each mutated residue has with its few neighboring residues in the interface. Note that the destabilization induced by TEM1 E104A is significantly greater than that due to the other mutations, as this residue interacts deep within the interface, although each mutation increases the dissociation rate relative to the wild type to some extent.

Conclusions

The rapid acquisition of accurate protein interaction data is a vital need in the investigation of many biological systems. The rapidly expanding field of proteomics, for example, demands reproducible, robust, high-performance methods to supplement traditional technology in the interrogation of the immense network of protein interactions in a cell. The ProteOn XPR36 protein interaction array system rapidly generates a 6 x 6 interaction array between six ligands and six analytes, and enhances studies of multiple protein interactions designed to map protein interfaces and resolve protein complexes. The example described here of an experimental design for analysis of multiple protein-protein interactions serves as a model for protocol development for the ProteOn XPR36 system.

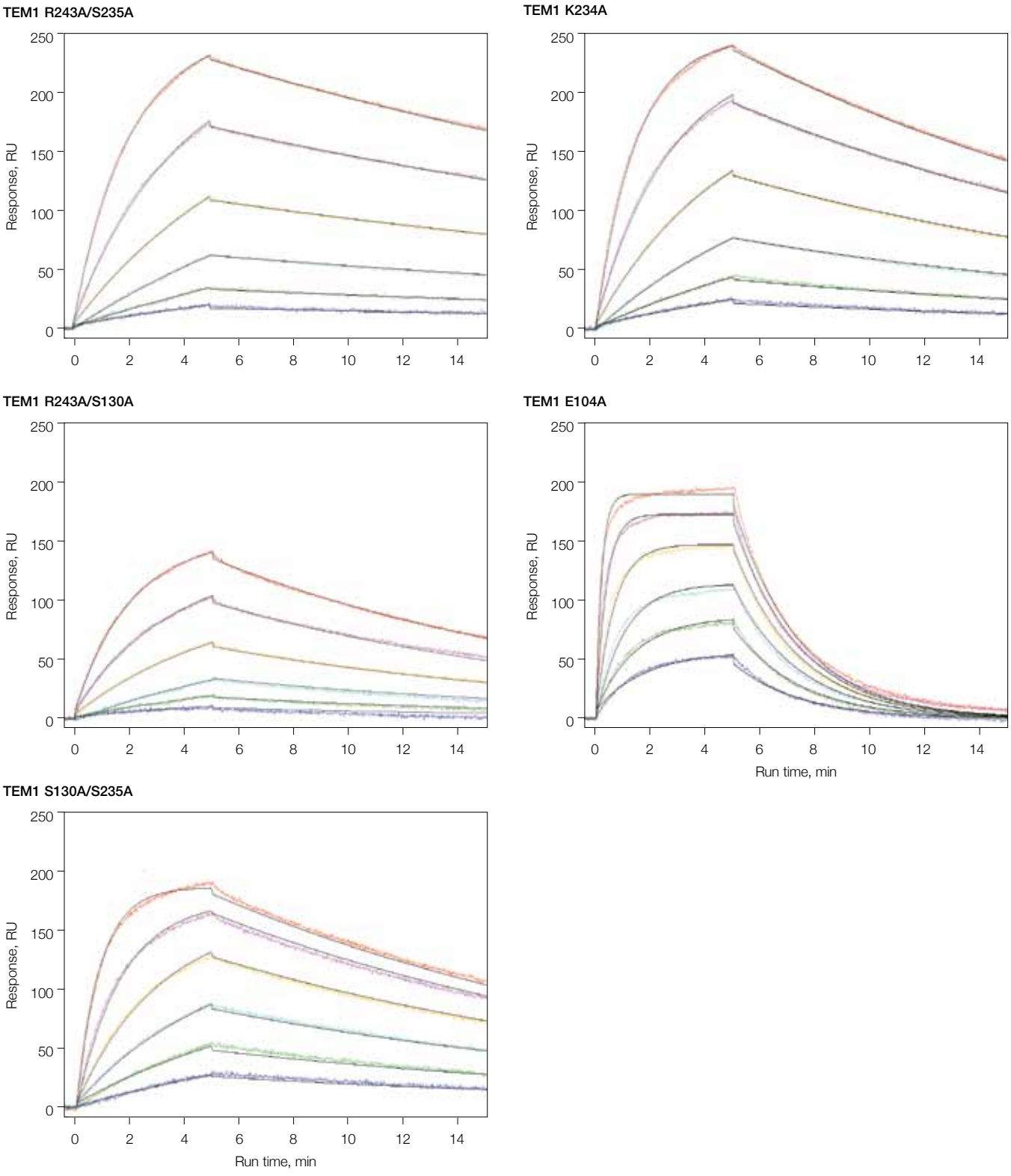

Fig. 2. One-shot kinetics for the interactions between BLIP and five TEM1 mutant proteins. Each set of six sensorgrams displays the responses from the six BLIP analyte concentrations (—, 600 nM; —, 300 nM; —, 150 nM; —, 75 nM; —, 37.5 nM; —, 18.75 nM) interacting with one (2 µM) TEM1 mutant protein ligand. Black lines represent the global fit of the sensorgrams to a 1:1 kinetic interaction model. See Table 1 for the kinetic constants derived from these data.

References

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