



Probing Receptor-Ligand Interactions Using Affinity Capillary Electrophoresis via a Multiple Injection Technique

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Abstract

This work utilizes a technique called multiple-injection affinity capillary electrophoresis (MIACE) to determine binding constants (K_b) between receptors and ligands using as model systems vancomycin and teicoplanin and their binding to D-Ala-D-Ala peptides and carbonic anhydrase B and its binding to arylsulfonamides. In a general form of the technique, a plug of sample containing a non-interacting standard is injected followed by multiple plugs of sample containing the receptor and then a final injection of sample containing a second standard. Between each injection of sample is injected a small plug of buffer containing an increasing concentration of ligand to effect separation between the multiple injections of sample. The electrophoresis is then carried out in an increasing concentration of ligand in the running buffer. Continued electrophoresis results in a shift in the migration time of the receptor in the sample plugs upon binding to their respective ligand yielding a value for K_b .

Introduction

Van (Figure 1A) from *Streptomyces orientalis* has been called the antibiotic of last resort because of its effectiveness in treating infections caused by bacteria resistant to other antibiotics. Like Van, teicoplanin (Teic) (Figure 1B) from *Actinoplanes teicomyceticus* and ristocetin (Rist) (Figure 1C) from *Nocardia lurida*, inhibit cell wall synthesis by impeding the action of transglycosylases and transpeptidases [1-3]. It is becoming increasingly important to develop new Van-group antibiotics, study their physicochemical parameters, and to examine their activity against van resistant enterococci (VRE). Of equal importance is the development of new analytical methods that would allow for high-throughput synthesis and binding analysis of potentially important drug targets. Current glycopeptide research focuses on one, the design of new antibiotics to better understand the factors which promote and hinder binding to their target molecules and, two, the development of new peptides to mimic surface modifications on the bacterial cell wall. In order to analyze these compounds, it is important to develop efficient analytical techniques to examine the effect the derivatization of glycopeptides and D-Ala-D-Ala terminus peptides have on the binding event.

Here we introduce multiple injection ACE (MIACE), a new analytical method of estimate binding constants of D-Ala-D-Ala terminus peptides to the glycopeptide antibiotics Teic, Rist, and Van (Figure 2). The data described here demonstrate the advantages of using MIACE to estimate binding parameters between ligands and antibiotics.

Results and Discussion

In the first set of experiments, we have examined the binding interactions between Vancomycin and D-Ala-D-Ala terminus peptides using MIACE. The column was charged with a sample containing the non-interacting marker (0.5 psi for 3 sec.) followed by five subsequent injections of Van (0.5 psi for 3 sec. each) (Figure 2) and electrophoresed. A representation of the electropherograms (Figure 3) will reveal that the peaks shift to the right due to the formation of the ligand-Van complex. Table one shows the binding constants that were obtained for each individual peak of Van. The scatchard plots clearly demonstrate the accuracy of this new MIACE technique. The technique was also used to determine the binding constant for Human CAB (Carbonic Anhydrase II), using CBSA (4-Carboxybenzenesulfonamide) as a ligand. The column was injected initially with two non-interacting plugs followed by multiple injections of CAB. Chromatograms (Figure 5) show a shift in CAB plugs revealing binding constants per peak (Table 2).

Conclusion

This report demonstrates the ease of using MIACE to determine binding constants between antibiotics and small peptides. Binding constants obtained by these results are in agreement with those values obtained in previous ACE techniques and traditional assay methods. This work establishes the feasibility of using novel ACE techniques to probe the binding between glycopeptide antibiotics and small peptides.

Acknowledgments

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Figure 1

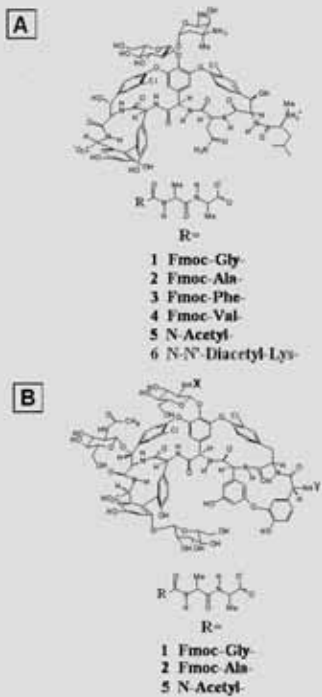


Figure 2



Figure 3

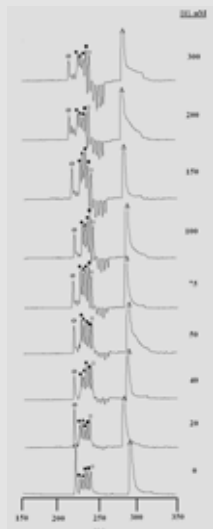


Figure 4

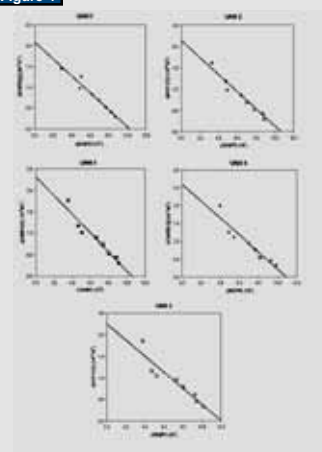


Table 1

1 to Van obtained by eq. 2.

Van Peak	K_b	R^2
1	21.4	0.950
2	22.5	0.960
3	23.0	0.922
4	25.1	0.890
5	19.6	0.871

Figure 5

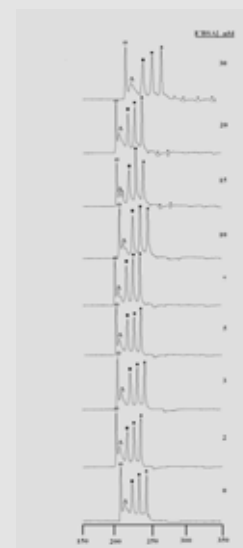


Table 2

Table 2. Experimental values of binding constants K_b ($10^6 M^{-1}$) of ligands CBSA to CAB

Ligand	K_b
CBSA	4.80 ^a

^aFluorescence excitation (342), $K_b = 0.77 \pm 0.2 \times 10^6 M^{-1}$ (pH 8.2).

Table 3

Table 3. Experimental values of binding constants K_b ($10^6 M^{-1}$) of ligands 1-5 to Van and Van obtained by eq. 2 and 1.

Antibiotic	Ligand	K_b
Van	1	18.0 ^a
	2	15.0 ^b
	3	21.0 ^c
	4	40.0
	5	3.0 ^d
Van	1	20.0 ^e
	2	40.0

^aFluorescence excitation (342), $K_b = 2.00 \times 10^6 M^{-1}$ (pH 8.0), $K_b = 21.8 \times 10^6 M^{-1}$ (pH 7.0).

^bFluorescence excitation (342), $K_b = 17.0 \times 10^6 M^{-1}$ (pH 7.0).

^cFluorescence excitation (342), $K_b = 16.0 \times 10^6 M^{-1}$ (pH 7.0).

^dFluorescence excitation (342), $K_b = 1.0 \pm 0.2 \times 10^6 M^{-1}$ (pH 8.0).