Anal Bioanal Chem (2004) 379 : 149–155
DOI 10.1007/s00216-004-2574-5

ORIGINAL PAPER

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Estimation of binding constants between ristocetin and teicoplanin and peptides using on-column ligand derivatization coupled to affinity capillary electrophoresis

Received: 21 November 2003 / Revised: 12 February 2004 / Accepted: 24 February 2004 / Published online: 24 March 2004
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Abstract This work utilizes on-column ligand synthesis and affinity capillary electrophoresis (ACE) to determine binding constants (K_b) of 9-flourenylmethyloxy carbonyl (Fmoc)-amino acid derivatives to the glycopeptide antibiotics ristocetin (Rist) and teicoplanin (Teic). In this technique, two separate plugs of sample are injected on to the capillary column and electrophoresed. The initial sample plug contains a D-Ala-D-Ala terminus peptide and either one or two non-interacting standard(s). The second plug contains a Fmoc-amino acid-*N*-hydroxysuccinimide (NHS) ester. The electrophoresis is then carried out with an increasing concentration of Rist or Teic in the running buffer. Upon electrophoresis the initial D-Ala-D-Ala peptide reacts with the Fmoc-amino acid yielding a new Fmoc-amino acid-D-Ala-D-Ala peptide derivative. Continued electrophoresis results in the binding of Rist or Teic to the Fmoc-amino acid-D-Ala-D-Ala peptide derivatives. Analysis of the change in the relative migration time ratio (*RMTR*) or electrophoretic mobility (μ) of the Fmoc-amino acid-D-Ala-D-Ala peptide derivatives relative to the non-interacting standards, as a function of the concentration of Rist and Teic, yields a value for K_b. These findings demonstrate the advantage of coupling on-column ligand synthesis to ACE for estimating binding parameters between antibiotics and ligands.

Keywords Ristocetin · Teicoplanin · Affinity capillary electrophoresis · Binding constants

Abbreviations *Rist* Ristocetin · *Teic* Teicoplanin · *ACE* Affinity capillary electrophoresis · *RMTR* Relative migration time ratio

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Introduction

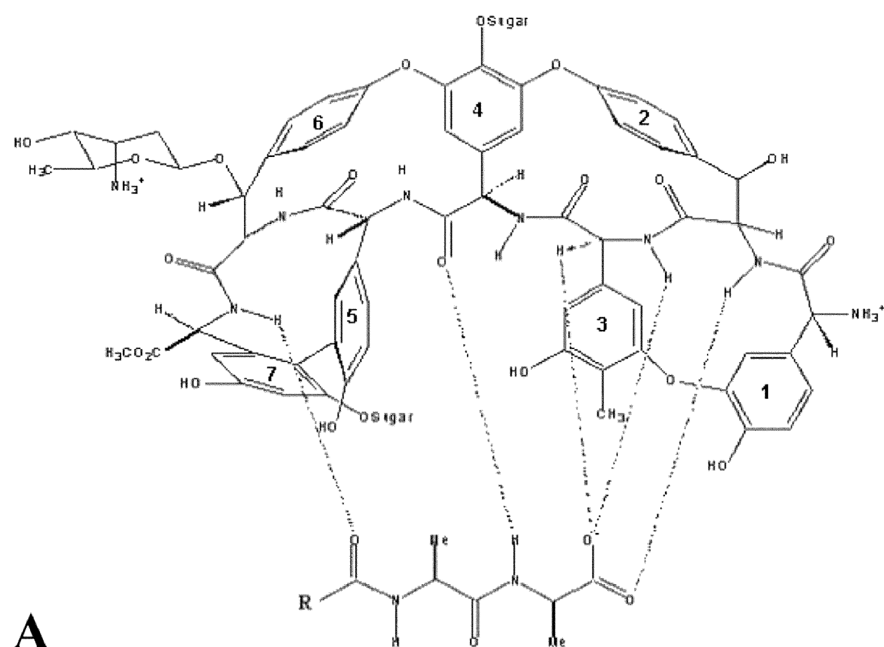
The development of resistance to antibacterial agents is a worldwide problem that continues to compromise the clinical effectiveness of drugs used in the treatment of many infectious diseases. Historically, glycopeptide antibiotics, and, in particular, vancomycin (Van) from *Streptomyces orientalis*, have been the standard class of molecules used to treat bacterial infections. Glycopeptide antibiotics inhibit the growth of Gram-positive bacteria by hindering cell wall peptidoglycan biosynthesis [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. These drugs bind to the D-Ala-D-Ala portion of peptidoglycan intermediates, thereby, inhibiting the transpeptidation reaction required for cross-linking of the cell wall [10]. This action of the glycopeptide antibiotic eventually causes bacterial cell death.

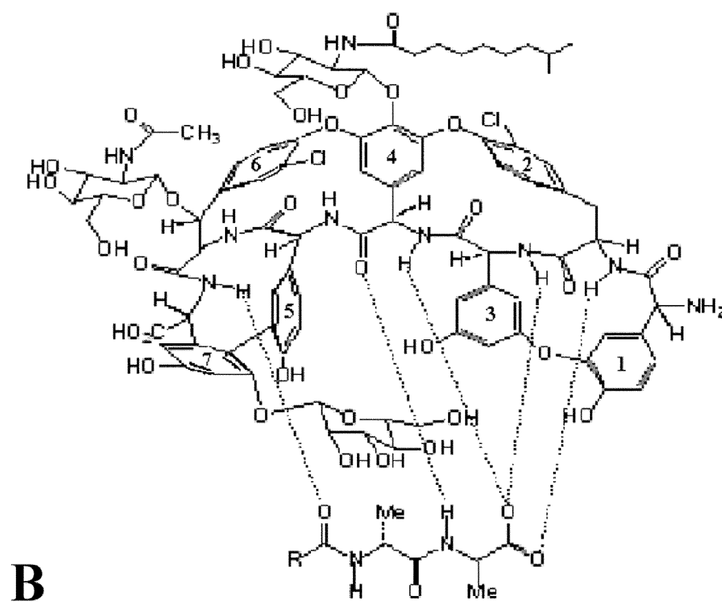
Ristocetin (Rist) (Fig. 1A) and teicoplanin (Teic) (Fig. 1B) are glycopeptide antibiotics of the Van group isolated from the microorganisms *Nocardia lurida* and *Actinoplanes teichomyceticus*, respectively [11]. They are composed of a linear heptapeptide, cross-linked between residues 1 and 3, 2 and 4, and 4 and 6 by diphenyl ether bridges and between residues 5 and 7 by a biphenyl bridge.

Based on NMR studies, a high specificity of binding between these glycopeptide antibiotics and D-Ala-D-Ala terminus peptides exist [12]. One of the main interactions is proposed to be a polar attraction between the carboxy group of the peptide and an amino or hydroxy group of the antibiotic in the hydrophobic environment of the complex. In the complex this polar interaction is insulated from the solvent by the peptide side chain. Hence, the effectiveness of this isolation depends on the length of the peptide and the size of the side chain in relation to the precise geometry of the antibiotic [11].

Rist exists in A and B forms differing only in one of the sugars. The sugar residues in Rist are not essential for antibiotic activity. Teic is a mixture of five closely related analogues, designated T-A_{1-1} through T-A_{1-5} [11]. They differ by approximately 20 molecular mass units because of the variation of the carbon length and substituent groups

Fig. 1 Structures of (**A**) ristocetin and (**B**) teicoplanin and the D-Ala-D-Ala ligands **1**–**8** used in this study





R

1 Fmoc-Gly-
2 Fmoc-Gly-D-Ala-
3 Fmoc-Gly-D-Ala-D-Ala-
4 Fmoc-Gly-Gly-Ala-Ala-
5 Fmoc-Ala-
6 Fmoc-Ala-D-Ala
7 Fmoc-Ala-D-Ala-D-Ala
8 Fmoc-Ala-Gly-Ala-Ala

of the hydrophobic acyl side chain (hydrophobic tail) that is attached to a 2-amino-2-deoxy-β-D-glucopyranosyl moiety [3]. This hydrophobic tail gives Teic its unique characteristics.

The greatest body of work involving glycopeptide antibiotics has focused on Van and its derivatives. Recently, this research demonstrated the positive effects hydrophobic derivatives of antibiotics have against glycopeptide-resistant Enterococci (GRE) [12, 13, 14]. There is limited binding constant information available for the interaction between Rist and Teic and D-Ala-D-Ala terminus peptides. Hence, there is a great need to determine binding affinities between these antibiotics and peptides.

During the past decade affinity capillary electrophoresis (ACE) has emerged as a useful and sensitive technique for studying bimolecular noncovalent interactions and for determining binding and dissociation constants of formed complexes [15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32]. ACE uses the resolving power of CE to distinguish between the free and bound forms of a receptor as a function of the concentration of free ligand. In a typical form of ACE, a sample of receptor and non-interacting standard(s) is exposed to an increasing concentration of ligand in the running buffer causing a shift in the migration time of the receptor relative to the standard(s). Subsequent analysis yields a value for the binding constant (K_b).

A number of interactions have been examined by ACE yielding a plethora of information on binding parameters. For example, Kaddis et al. showed that binding constants of neutral ligands to carbonic anhydrase B could be estimated using ACE [33]. Qian et al. used ACE to investigate an epitope on human immunodeficiency virus by a monoclonal antibody [23]. Finally, Kessig et al. used ACE to examine the interaction of the enzyme cyclophilin with the immunosuppressive drug cyclosporin A [34].

We recently described a two-step procedure, whereby, on-column ligand synthesis and partial-filling affinity capillary electrophoresis (PFACE) were coupled to each other to determine binding constants of peptides to Van [15]. In these studies, differential electrophoretic mobilities were utilized to merge separate zones of analyte and reagent(s) under the influence of an electric field to form product. Continued electrophoresis resulted in zones of product(s) and Van overlapping where a dynamic equilibrium was established. Subsequently, a binding constant was estimated by observing the change in migration time of the peptide at different concentrations of Van. We extended this work by derivatizing the antibiotics teicoplanin and ristocetin using on-column techniques [18]. In order to further demonstrate the versatility of on-column derivatization coupled to ACE in open tubular capillary columns it is important to examine other receptor–ligand systems.

Herein, we extend our work on-column derivatization of D-Ala-D-Ala terminus peptides and ACE by determining binding constants of Rist and Teic to peptides. In this technique an increasing concentration of antibiotic in the running buffer is used to create a shift in migration time of the derivatized peptide on complexation to the antibiotic. Subsequent Scatchard analysis yields a value for K_b. The values for the binding constants are correlated to the hydrophobicity of the peptide and the antibiotic.

Methods

Chemicals and Reagents

All chemicals were analytical grade. Ristocetin was purchased from Bio Data Corporation (Horsham, PA, USA) and was used without further purification. Teicoplanin·HCl was purchased from Advance Separation Technologies (Whippany, NJ, USA) and was used without further purification. D-Ala-D-Ala, D-Ala-D-Ala-D-Ala, D-Ala-D-Ala-D-Ala-D-Ala, Gly-Ala-Ala-D-Ala-D-Ala, benzenesulfonamide (BSA), and 4-carboxybenzenesulfonamide (CBSA) were purchased from Sigma (St Louis, MO, USA) and were used without further purification. Fmoc-Gly-NHS and Fmoc-Ala-NHS were purchased from Bachem California (Torrance, CA, USA). Stock solutions of CBSA ($0.4\,g\,L^{-1}$), D-Ala-D-Ala ($0.1\,g\,L^{-1}$), D-Ala-D-Ala-D-Ala ($0.1\,g\,L^{-1}$), D-Ala-D-Ala-D-Ala-D-Ala ($0.1\,g\,L^{-1}$), and Gly-Ala-Ala-D-Ala-D-Ala ($0.1\,g\,L^{-1}$) were each prepared by dissolving in buffer ($20\,mmol\,L^{-1}$ phosphate buffer; pH 7.5). Stock solutions of Fmoc-Gly-NHS ester ($2\,g\,L^{-1}$) and Fmoc-Ala-NHS ester ($4\,g\,L^{-1}$) were prepared by dissolving the compound in acetonitrile.

Apparatus

The capillary electrophoresis (CE) system used in this study was a Beckman Model P/ACE 5510 (Fullerton, CA, USA). The capillary tubing (Polymicro Technology, Phoenix, AZ, USA) used for the experiment was uncoated fused silica with an internal diameter of 50 μm, length from inlet to detector of 40.5 cm, and a length from detector to outlet of 6.5 cm. The conditions used in CE were as follows: voltage, 24 kV; current, 35 μA; detection, 205 nm; temperature, 23.0±0.1 °C. Data were collected and analyzed with Beckman System Gold software.

Procedures

For on-column ligand synthesis coupled to ACE: the capillary was first equilibrated with buffer ($20\,mmol\,L^{-1}$ phosphate buffer; pH 7.5) containing increasing concentration of antibiotic ($0–100\,\mu mol\,L^{-1}$ for Rist; $0–60\,\mu mol\,L^{-1}$ for Teic) for 1 min. Separate plugs of sample solution (1.2 nL) containing D-Ala-D-Ala terminus peptide and non-interacting markers (CBSA and/or BSA), Fmoc-amino acid-NHS ester in acetonitrile (1.2 nL), were introduced by pressure injection. The electrophoresis was carried out in phosphate buffer with increasing concentration of the antibiotic and run at 24 kV for 5.0 min to complete the detection of all species.

Results and discussion

The sample of Rist used in this study was a mixture of two structurally similar compounds differing by the number of carbons in one of its sugar moieties. The Rist mixture is >95% Rist A (Fig. 1A). The Teic sample used was a mixture of five closely related compounds differing by the number of carbons and substituted groups attached to the fatty acid side chain attached to the amino sugar (Fig. 1B).

In the first series of experiments, we examined the binding of D-Ala-D-Ala-D-Ala to Rist using an on-column ligand synthesis coupled to ACE technique. A multi-plug injection technique was utilized in this method. After equilibrating

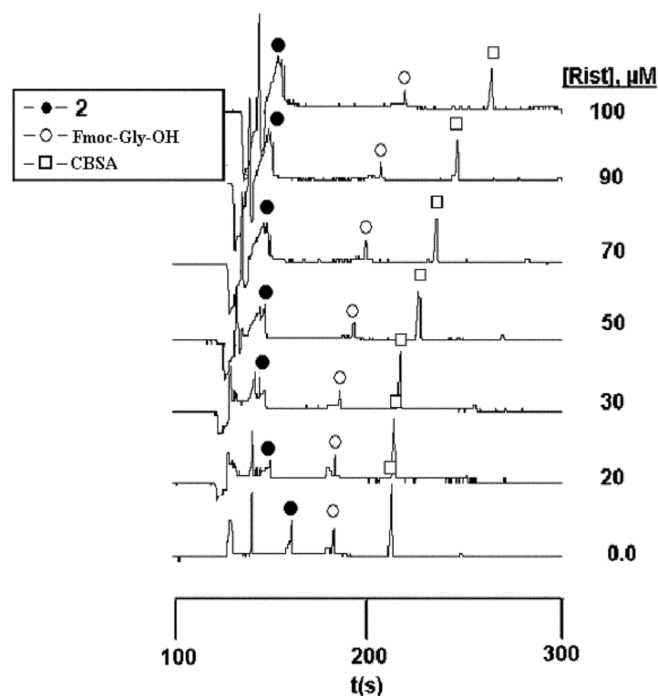


Fig. 2 A representative set of electropherograms of **2** in 20 mmol L^{-1} phosphate buffer (pH 7.5) containing different concentration of Rist, obtained by affinity capillary electrophoresis. The total analysis time in each experiment was 5 min at 24 kV (current 34–35 μA) using a 30.5-cm (inlet to detector), 50-μm i.d. open, uncoated quartz capillary. CBSA was used as internal standard

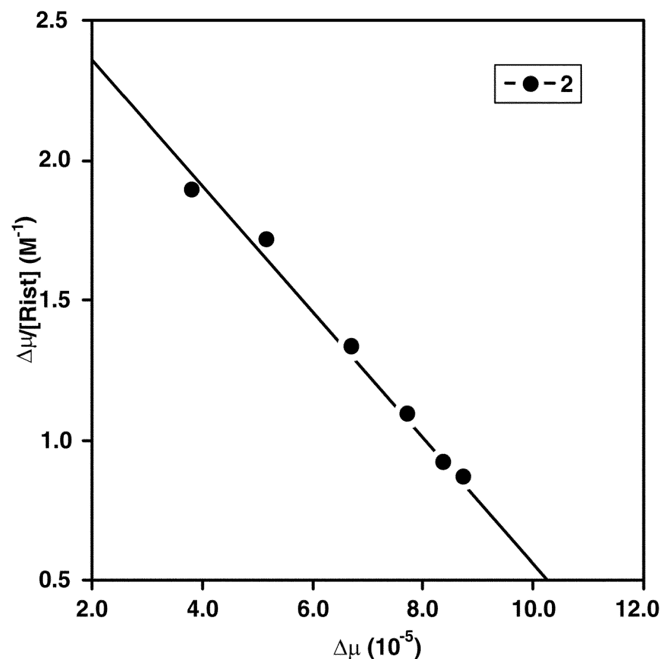


Fig. 3 Scatchard plot of the data for **2** and Rist according to Eq. (2)

the capillary with an increasing concentration of Rist in phosphate buffer solution, plugs of sample containing D-Ala-D-Ala-D-Ala and a non-interacting standard (CBSA) is vacuum injected, followed by a plug of Fmoc-Gly-NHS ester in acetonitrile. The sample plugs are electrophoresed in increasing concentration of Rist. Overlap of the separate zones of species yields Fmoc-Gly-D-Ala-D-Ala-D-Ala (**2**). Although the Fmoc reagent was dissolved in acetonitrile to lessen hydrolysis, small amounts of hydrolyzed product (Fmoc-Gly-OH) were identified in the electropherogram. The hydrolysis product is, therefore, used as the second non-interacting standard, since it has no binding affinity for the antibiotic and does not vary in migration time from run to run. Continued electrophoresis allows for the zone of **2** and the solution of Rist to overlap forming a dynamic equilibrium between the two species.

Figure 2 shows a representative series of electropherograms of **2** in a capillary filled with increasing concentrations of Rist at 205 nm. As the concentration of Rist was increased (0 to 100 μmol L^{-1}) in the running buffer the peak for **2** shifted to a shorter migration time. The height of the peak for **2** increases on complexation to Rist due to the higher combined absorbance of the complex in comparison to **2** alone. We estimated the yield of the reaction (formation of **2**) to be approximately 30% based on the amount of side-products (Fmoc-Gly-OH) and starting materials (D-Ala-D-Ala-D-Ala and Fmoc-Gly-NHS).The area of the peaks was constant over multiple runs within a given concentration of Rist. We, therefore, assumed a similar amount of **2** was synthesized throughout the duration of the experiment. The efficiency of peptide derivatization varies depending on the stoichiometry of the reagents. In the present work we have utilized a constant amount of reagents and find, that in any given electrophoresis run, a similar amount of product is formed. A 100% conversion to product was never observed. We believe the reason for this is: one, peptide derivatization cannot fully compete with the hydrolysis of the NHS ester, and; two, that there is insufficient time for the peptide reaction to occur while in the capillary column. Fortunately, complete conversion to product is not required; only constant conversion from one run to the next.

Upon complexation of **2** to Rist the **2**-Rist complex is less negatively charged and has an increase in mass and elutes from the column faster than the uncomplexed form. At pH 8.3 Rist is slightly positively charged which causes a shift in the migration time of **2** (as the **2**-Rist complex) to shorter times. Some peak broadening was observed at intermediate concentrations of Rist. This is caused by the retardation of migrating molecules due to their frequent interactions with the ligand in the region of intermediate status [15]. The inverted peak observed above [Rist] = 50 μmol L^{-1} is due to the dilution of Rist in the running buffer upon complexation to **2**. Figure 3 is a Scatchard plot of the data for **2**.

Two forms of analysis, one using a single marker and the other using two markers were used to estimate K_b. In the single marker form of analysis K_b is estimated using one non-interacting standard which relates changes in the electrophoretic mobility ($\mu_{P,A}$) of the D-Ala-D-Ala terminus peptide on complexation with the antibiotic (A) pre-

sent in the buffer to K_b. Analysis of the magnitude of the change in mobility, $\Delta\mu_{P,A}$, as a function of the concentration [A] of receptor yields K_b (Eq. 1):

$$\Delta\mu_{P,A} = \mu_{P,A} - \mu_P = l_c l_d / V\left[(1/t_{P,A} - 1/t_{m,A}) - (1/t_P - 1/tm)\right] \quad (1)$$

where $\Delta\mu_{P,A}$ is the change in mobility of the peptide as a function of the concentration of Rist, $t_{P,A}$ and $t_{m,A}$ are the measured migration times of the sample peak (**2**) and the non-interacting standard (CBSA) at the concentration of antibiotic, respectively, l_c (cm) is the total length of the capillary, l_d (cm) is the length of capillary from the inlet end of the capillary to the detector, t_m (s) is the measured migration time of the non-interacting standard, t_P is the measured migration time of the D-Ala-D-Ala terminus peptide, and V is the voltage across the capillary. The values of $\Delta\mu_{P,A}$ obtained using Eq. (1) over a range of concentrations of Rist may then be used for Scatchard analysis (Eq. 2):

$$\Delta\mu_{P,A} / [A] = K_b \Delta\mu_{P,A}^{max} - K_b \Delta\mu_{P,A} \quad (2)$$

Equation (2) allows for the estimation of K_b on a relative time scale using one non-interacting standard. This equation compensates for changes in electroosmotic flow (EOF) in the capillary column induced by variations in viscosity and/or Joule heating. Table 1 summarizes the binding data obtained for the binding of glycopeptides to D-Ala-D-Ala terminus peptides. The values for K_b obtained by the on-column ligand derivatization technique are comparable to those observed using standard ACE techniques [16, 19].

In the second form of analysis, K_b is estimated using two non-interacting standards, which we term the relative migration time ratio (*RMTR*) (Eq. 3) [25].

$$RMTR = (t_p - t_s') / (t_s' - t_s) \quad (3)$$

Here, t_p, t_s, and t_s' are the measured migration times of the peptide peak, and two non-interacting standard peaks (CBSA and Fmoc-Gly-OH) respectively. A Scatchard plot can be obtained via Eq. (4):

$$\Delta RMTR_{P,A} / [L] = K_b \Delta RMTR_{P,A}^{max} - K_b \Delta RMTR_{P,A} \quad (4)$$

Table 1 Experimental values of binding constants K_b (10^4 mol L^{-1}) of receptors **1–8** to Rist and Teic obtained by use of Eqs (2) and (4)

Peptide	Rist K_b (correlation coefficient)	Teic K_b (correlation coefficient)
Fmoc-Gly-(D-Ala)$_2$	0.82 (0.95)	2.18 (0.93)
Fmoc-Gly-(D-Ala)$_3$	2.19 (0.99)	1.79 (0.96)
Fmoc-Gly-(D-Ala)$_4$	2.96 (0.97)	16.51 (0.99)
Fmoc-(Gly)$_2$-(Ala)$_2$-(D-Ala)$_2$	2.40 (0.95)	7.58 (0.93)
Fmoc-Ala-(D-Ala)$_2$	5.24 (0.95)	18.51 (0.99)
Fmoc-Ala-(D-Ala)$_3$	5.18 (0.97)	13.72 (0.97)
Fmoc-Ala-(D-Ala)$_4$	4.64 (0.96)	4.42 (0.99)
Fmoc-Ala-Gly-(Ala)$_2$-(D-Ala)$_2$	4.72 (0.96)	3.50 (0.98)

The binding constants are the average values of four repetitions for each ligand concentration

Here $\Delta RMTR_{P,A}$ is the magnitude of change in the *RMTR* as a function of the concentration of antibiotic. Equation (4) allows for the estimation of K_b on a relative time scale using two non-interacting standards. Similar to Eq. (2), Eq. (4) also corrects for changes in EOF that occur in the capillary column due to variations in viscosity and Joule heating.

In the second series of experiments, we examined the binding of D-Ala-D-Ala terminus peptides to Teic using the same technique as the first series of experiments with Rist. The same multi-plug injection technique was utilized in the experiment with Rist. After equilibrating the capillary with an increasing concentration of Teic in phosphate buffer solution, a plug of sample is injected containing D-Ala-D-Ala terminus peptide and a non-interacting standard. A sample plug of Fmoc-amino acid-NHS ester in acetonitrile is then injected. The sample plugs are electrophoresed in increasing concentrations of Teic. Overlap of the separate zones of species yields the new Fmoc-Gly-D-Ala-D-Ala-D-Ala (**2**) species. Continued electrophoresis allows the zone of Teic to migrate into the zone of **2,** thereby forming a dynamic equilibrium between Teic and **2**.

Figure 4 shows a representative series of electropherograms of **2** in a capillary filled with increasing concentrations of Teic at 205 nm. As the concentration of Teic was increased (0 to 60 µmol L^{-1}) in the running buffer **2** shifts to the left (shorter migration time). Due to the greater mass of the newly formed complex upon increasing concentration of Teic the height of the peak for **2** increases on increasing concentration of Teic. This increase in peak height (and area) is commonly observed in ACE studies when

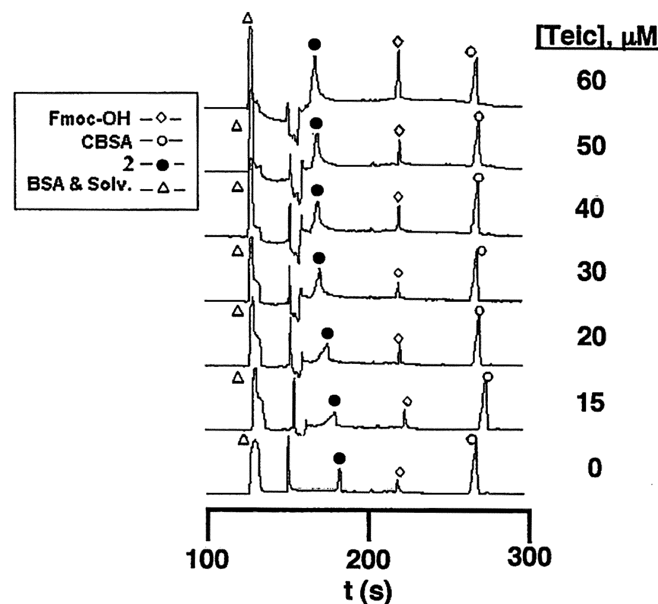


Fig. 4 A representative set of electropherograms of **2** in 20 mmol L^{-1} phosphate buffer (pH 7.5) containing different concentrations of Teic, obtained by affinity capillary electrophoresis. The total analysis time in each experiment was 5 min at 24 kV (current 34–35 µA) using a 30.5-cm (inlet to detector), 50-µm i.d. open, uncoated quartz capillary. CBSA and Fmoc-OH were used as internal standards

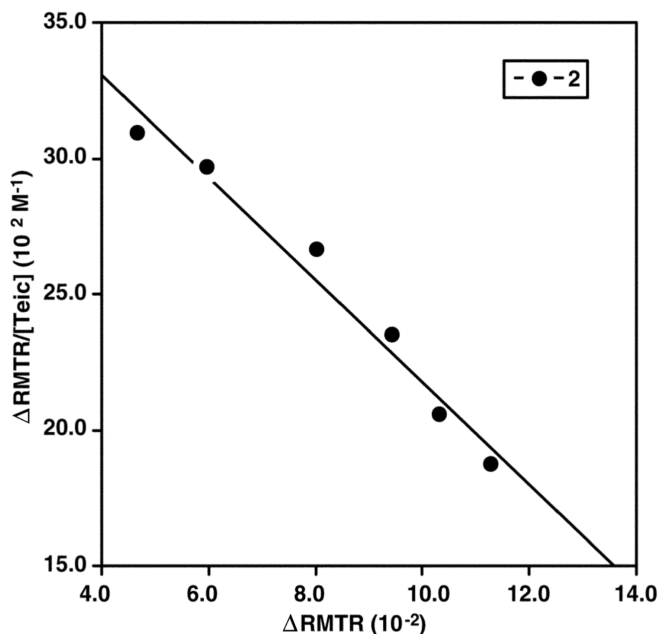


Fig. 5 Scatchard plot of the data for **2** and Teic according to Eq. (4)

the peak of interest is of smaller molecular weight and/or is less chromophoric (or has a smaller extinction coefficient) than the species whose concentration is being changed in the running buffer. Figure 5 is a Scatchard plot of the data for **2**.

Table 1 summarizes the binding data obtained for D-Ala-D-Ala terminus peptides to Rist and Teic. Both Eqs (2) and (4) were used to calculate the binding constants. Both methods compensate for changes in EOF and can be used in ACE analysis. We obtained binding constants for eight D-Ala-D-Ala terminus peptides. The values for K_b between Fmoc-Ala-peptides and Rist are greater than the Fmoc-Gly-peptides. The values obtained for K_b closely correlate to previously reported K_b values of D-Ala-D-Ala terminus peptides to Teic obtained by either ACE or other binding assays. Fmoc-Ala-peptides possess an extra methyl group in comparison to the Fmoc-Gly-peptides. These results support the hypothesis that peptides with longer side chains better insulate the hydrophobic pocket of glycopeptide antibiotics where binding to D-Ala-D-Ala terminus peptides occur. Hence, greater binding constants are realized for these peptides.

This same observation was not found for values of K_b of peptides to Teic. These results may be due to differences in the binding pockets of the two antibiotics. Teic appears to have the smallest binding pocket (~4.5–5.5 Å) among the family of Van glycopeptide antibiotics. Rist has a larger binding pocket (~5.2–8.8 Å) [3]. The Teic results suggest that the carboxy terminus of Fmoc-amino acids is the initial contact to the antibiotic binding pocket. Peptides **5** and **6** displayed higher binding affinity to Teic than peptides **1** and **2**. These differences can best be explained by the size of the side chain on the peptide. Peptides with Fmoc-Ala yielded higher values for K_b. This trend is reversed with longer Fmoc-amino acids, vis-à-vis comparing **7** to **3** and **8** to **4**.

The results show that, in general, Teic has a higher affinity to D-Ala-D-Ala terminus peptides than Rist. Teic has one unique characteristic: it has a hydrophobic acyl side chain (" hydrophobic tail") attached to a 2-amino-2-deoxy-β-D-glucopyranosyl moiety (Fig. 1A). It has been hypothesized that the hydrophobic tail of Teic, in situ, functions as a membrane anchor [6]. It is believed that the hydrophobic side chain interacts with the membrane to anchor Teic at the target site. This process facilitates the binding of Teic to the D-Ala-D-Ala terminus peptide of the growing bacterial membrane [6]. Whether this distinguishing characteristic of Teic is reason for its higher affinity to the D-Ala-D-Ala terminus peptides, in vitro, has yet to be proven.

We have demonstrated that ACE is a versatile method to accurately study bimolecular noncovalent interactions. One important advantage of this technique is that small quantities of receptor and ligand are needed to conduct the studies in comparison to traditional methods. Purification of the sample prior to injection is not necessary as long as the component to be analyzed can be separated from other species. Finally, the commercial availability of automated instrumentation, and high reproducibility of data, make it experimentally convenient.

Conclusions

The present study demonstrates the ease of using on-column ligand derivatization coupled to ACE to determine binding constants between antibiotics and small peptides. In this work D-Ala-D-Ala terminus peptides were derivatized with Fmoc-amino acid esters and their binding to Teic and Rist examined. In this method, the capillary is filled with peptile, derivatizing reagent, complexing molecule and electrophoresed. Derivatization occurs upon overlap of separate zones of solution yielding product which, upon electrophoresis, interacts with the antibiotic injected in the capillary at increasing concentrations. Binding constants are then estimated using changes in migration time of the peptide. The binding constants obtained by these techniques are in agreement with values obtained using previous ACE techniques and traditional assay methods.

A major reason for conducting this work was to further demonstrate the efficacy of using on-column ligand derivatization coupled to ACE in the estimation of binding constants. While this work once again employs glycopeptide antibiotics–peptides as the receptor–ligand system it singly exemplifies the power and ease of conducting microscale reactions within the capillary and the subsequent measurement of affinity between receptor and ligand. The novelty lies in generating derivatized peptides on-column without the need of precolumn derivatization and/or purification steps. The results provided herein therefore broaden the appeal of CE as a versatile analytical technique in wide-ranging fields and especially in the development and design of drug targets in the pharmaceutical industry.

Acknowledgments The authors gratefully acknowledge financial support for this research by grants from the National Science Foundation (CHE-0136724), Research Corporation (CC5293), the Department of Defense (F49620-02-1-0445), and the National Institutes of Health (R15 AI055515-01).

References

1. Allen NE, LeTourneau DL, Hobbs JN (1997) Antimicrob Agents Chemother 41:66–71
2. Heck AJR, Bonnici PJ, Breukink E, Morris D, Wills M (2001) Chem Eur J 7:910–916
3. Arimoto H, Nishimura K, Kinumi T, Hayakawa I, Uemura D (1999) Chem Commun 1361–1362
4. Beauregard DA, Maguire AJ, Williams DH, Reynolds PE (1997) Antimicrob Agents Chemother 41:2418–2423
5. Chiosis G, Boneca IG (2001) Science 293:1484–1487
6. Kerns R, Dong SD, Fukuzawa S, Carbeck J, Kohler J, Silver L, Kahne D (2000) J Am Chem Soc 122:12608–12609
7. Dancer RJ, Try AC, Sharman GJ, Williams DH (1996) J Chem Soc Chem Commun 12:1445–1446
8. Noble WC, Virani Z, Cree RGA (1992) FEMS Microbiol Lett 93:195–198
9. Barna JCJ, Williams DH (1984) Ann Rev Microbiol 38:339–357
10. Perkins HR (1969) Biochem J 111:195–205
11. Kim KH, Martin Y, Otis E, Mao J (1989) J Med Chem 32:84–93
12. Malabarba A, Nicas TI (1997) Med Res Rev 17:69–137
13. Pavlov AY, Preobrazhenskaya MN (1998) Russ J Bioorg Chem 24:570–587
14. Printsevaskaya SS, Pavlov AY, Olsufyeva EN, Mirchink EP, Isakova EB, Reznikova M I, Goldman RC, Branstorm AA, Baizman ER, Longley CB, Sztaricskai F, Batta G, Preobrazhenskaya MN (2002) J Med Chem 45:1340–1347
15. Zhang Y, Kodama C, Zurita C, Gomez FA (2001) J Chromatogr A 928:233–241
16. Silverio CF, Plazas A, Moran J, Gomez FA (2002) J Liq Chromatogr Related Technol 25:1677–1691
17. Progent F, Taverna M, Le Potier I, Gopee F, Ferrier D (2002) Electrophoresis 23:938–944
18. Silverio CF, Azad M, Gomez FA (2003) Electrophoresis 24:808–815
19. Azad M, Hernandez L, Plazas A, Rudolph M, Gomez FA (2003) Chromatographia 57:339–343
20. Kraak JC, Busch S, Poppe H (1992) J Chromatogr 608:257–264
21. Liu J, Volk KJ, Lee MS, Pucci M, Handwerger S (1994) Anal Chem 66:2412–2416
22. Chu Y-H, Dunayevskiy YM, Kirby DP, Vouros P, Karger BL (1996) J Am Chem Soc 118:7827–7835
23. Qian X-H, Tomer KB (1998) Electrophoresis 19:415–419
24. Lin S, Hsiao IY, Hsu SM (1997) Anal Biochem 254:9–17
25. Mito E, Zhang Y, Esquivel S, Gomez FA (2000) Anal Biochem 280:209–215
26. Heintz J, Hernandez M, Gomez FA (1999) J Chromatogr A 840:261–268
27. Gomez FA, Mirkovich JN, Dominguez VM, Liu KW, Macias DM (1996) J Chromatogr A 727:291–299
28. Zhao DS, Kwak E-S, Kawaoka J, Esquivel, S Gomez FA (1998) Am Lab 40–48
29. Colton JJ, Carbeck JD, Rao J, Whitesides GM (1998) Electrophoresis 19:367–382
30. Rundlett KL, Armstrong DW (1997) Electrophoresis 18:2194–2202
31. Meisel H, Olieman C (1998) Anal Chim Acta 372:291–297
32. Abler JK, Reddy KR, Lee CS (1997) J Chromatogr A 759:139–148
33. Kaddis J, Mito E, Heintz J, Plazas A, Gomez FA (2003) Electrophoresis 24:1105–1110
34. Kiessig S, Bang H, Thunecke F (1999) J Chromatogr A 853:469–477

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