ELSEVIER

Journal of Chromatography A, 928 (2001) 233–241

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

On-column ligand synthesis coupled to partial-filling affinity capillary electrophoresis to estimate binding constants of ligands to a receptor

Ying Zhang, Cynthia Kodama, Cecilia Zurita, Frank A. Gomez*

Department of Chemistry and Biochemistry, California State University, Los Angeles, 5151 State University Drive, Los Angeles, CA 90032-8202, USA

Received 28 December 2000; received in revised form 6 July 2001; accepted 9 July 2001

Abstract

This paper describes a two-step procedure whereby on-column ligand synthesis and partial-filling affinity capillary electrophoresis (PFACE) are sequentially coupled to each other to determine the binding constants of 9-fluorenylmethoxy carbonyl (Fmoc)-amino acid–D-Ala–D-Ala species to vancomycin (Van) from *Streptomyces orientalis*. In this technique four separate plugs of sample are injected onto the capillary column and electrophoresed. The initial sample plug contains a D-Ala–D-Ala terminus peptide and two non-interacting standards. Plugs two and three contain solutions of Fmoc-amino acid–*N*-hydroxysuccinimide (NHS) ester and running buffer, respectively. The fourth sample plug contains an increasing concentration of Van partially-filled onto the capillary column. Upon electrophoresis the initial D-Ala–D-Ala peptide reacts with the Fmoc-amino acid NHS ester yielding the Fmoc-amino acid D-Ala–D-Ala peptide. Continued electrophoresis results in the overlap of the plugs of Van and Fmoc-amino acid–D-Ala–D-Ala peptide and non-interacting markers. Analysis of the change in the relative migration time ratio of the Fmoc-amino acid–D-Ala–D-Ala peptide relative to the non-interacting standards, as a function of the concentration of Van, yields a value for the binding constant. These values agree well with those estimated using other binding and ACE techniques.

Keywords: Affinity capillary electrophoresis; Partial filling capillary electrophoresis; Binding constants; Ligand synthesis, on column; *Streptomyces orientalis*; Vancomycin; Peptides

1. Introduction

Capillary electrophoresis (CE) has become an important analytical technique in many research laboratories primarily because of its small sample size requirement and high speed of analysis [1]. CE separates molecules based on their charge-to-mass ratio upon application of a voltage gradient. During the past decade a number of versatile CE techniques have been developed to explore the physical biochemistry of biomolecules. Of these techniques enzyme-mediated microanalysis (EMMA) [2–13] and affinity capillary electrophoresis (ACE) [14–36] have shown great promise in the analysis of biophysical parameters.

EMMA has been used to monitor on-column reactions in particular enzyme-catalyzed microreactions. For example, Zugel et al. used an on-column

*Corresponding author. Tel.: +1-323-343-2368; fax: +1-323-343-6490.

E-mail address: fgomez2@calstatela.edu (F.A. Gomez).

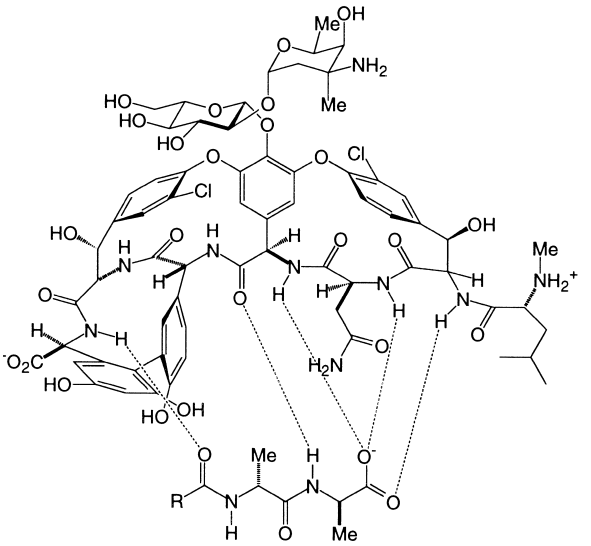
0021-9673/01/$ – see front matter
PII: S0021-9673(01)01117-7

technique to assay leucine aminopeptidase on a microchip [2]. Jin et al. used an on-column approach to monitor the oxidation of glucose by glucose oxidase in submicroliter samples [3]. Finally, Kwak et al. related electrophoresis conditions of in-capillary enzyme-catalyzed microreactions to product distribution profiles [4]. In these studies differential electrophoretic mobility is utilized to merge zones of analyte and reagent(s) under the influence of an electric field. Upon electrophoresis zones of sample overlap yielding product(s) which is then transported to the detector.

Concurrent with the development of EMMA has been the use of ACE to examine binding parameters of receptors to ligands. For example, Kiessig et al. used ACE to examine the interaction of the enzyme cyclophilin with the immunosuppressive drug cyclosporin A [15]. Gao et al. has examined protein charge ladders by ACE to determine the contribution of electrostatics to the values of measured binding affinities [16]. Finally, Qian et al. used ACE to investigate an epitope on human immunodeficiency virus recognized by a monoclonal antibody [17]. ACE uses the resolving power of CE to distinguish between free and bound forms of a receptor as a function of concentration of free ligand. In a traditional ACE study increasing concentrations of ligand in the running buffer cause a shift in the migration time of the receptor. Analysis of this change in migration time relative to a standard(s) yields a binding constant.

Recently, our laboratories [33,34] and others [35,36] developed partial-filling ACE (PFACE) techniques to analyze receptor–ligand interactions. These techniques have greatly simplified the use of ACE by expediting the binding experiment and reducing the amount of material required in the assay. In this technique, the capillary is partially-filled with a ligand followed by a sample of receptor and non-interacting standards. Upon electrophoresis zones of reagent overlap creating a dynamic equilibrium between ligand and receptor within the capillary column. A binding constant can then be obtained by examining the change in the migration time of the receptor referenced to the internal standards.

Vancomycin (Van) is a parenteral glycopeptide antibiotic that kills bacterial cells by inhibiting peptidoglycan biosynthesis (Fig. 1). It functions by binding to the terminal D-Ala–D-Ala dipeptide of the bacterial cell wall precursors thereby impeding further processing of these intermediates into peptidoglycan [37]. Van is particularly useful against penicillin- and methicillin-resistant staphylococcal infections and for treating gram-positive infections in penicillin-allergic patients. Extensive studies on molecular recognition between Van and various small peptides with D-Ala–D-Ala terminus and structurally modified variants have been conducted by a number of techniques realizing binding constants in the 10^3–10^5 M^{-1} range [16–21,28,36,38–40].



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-D-Ala-D-Ala-
6 Fmoc-Ala-
7 Fmoc-Ala-D-Ala-
8 Fmoc-Ala-Gly-Ala-Ala-

Fig. 1. Vancomycin and D-Ala–D-Ala ligands **1–8** used in this study.

In recent years the development of new drugs has been almost exponential in growth due to new synthetic and analytical approaches to rational drug design. This increased output of potential drugs

compared to traditional techniques to drug design has made expeditious and facile analysis of new drugs a must in any new analytical technique. Recently, ACE was used to examine the binding affinities of ligands to receptors resulting from a high throughput screening and combinatorial approach to rational drug design [18–21,23,30,41–43]. However, these approaches examined ligands to receptors that were synthesized off-column and prior to analysis by CE. To date, no study has documented the coupling of on-column synthetic techniques and ACE in the analysis of binding parameters. A method that would allow for high throughput analysis coupled to ligand synthesis would expedite analysis of potential molecular targets.

Herein, we describe a two-step procedure whereby 9-flourenylmethoxy carbonyl (Fmoc)-amino acid–D-Ala–D-Ala ligands are synthesized on-column and subsequently analyzed for their affinity to Van using PFACE (Fig. 2). In this technique separate plugs of peptide(s) and non-interacting markers, Fmoc-amino acid–NHS ester reagent, buffer, and Van are injected onto the capillary column and electrophoresed. Peptide and Fmoc-amino acid–*N*-hydroxysuccinimide (NHS) ester overlap upon electrophoresis forming the new Fmoc-derivatized peptide. The zone of Van then overlaps with the new Fmoc-peptide thereby inducing a change in its electrophoretic mobility on increasing concentrations of Van in the partially-filled column. Analysis of the change in the relative migration time ratio (RMTR) yields a value for the binding constant.

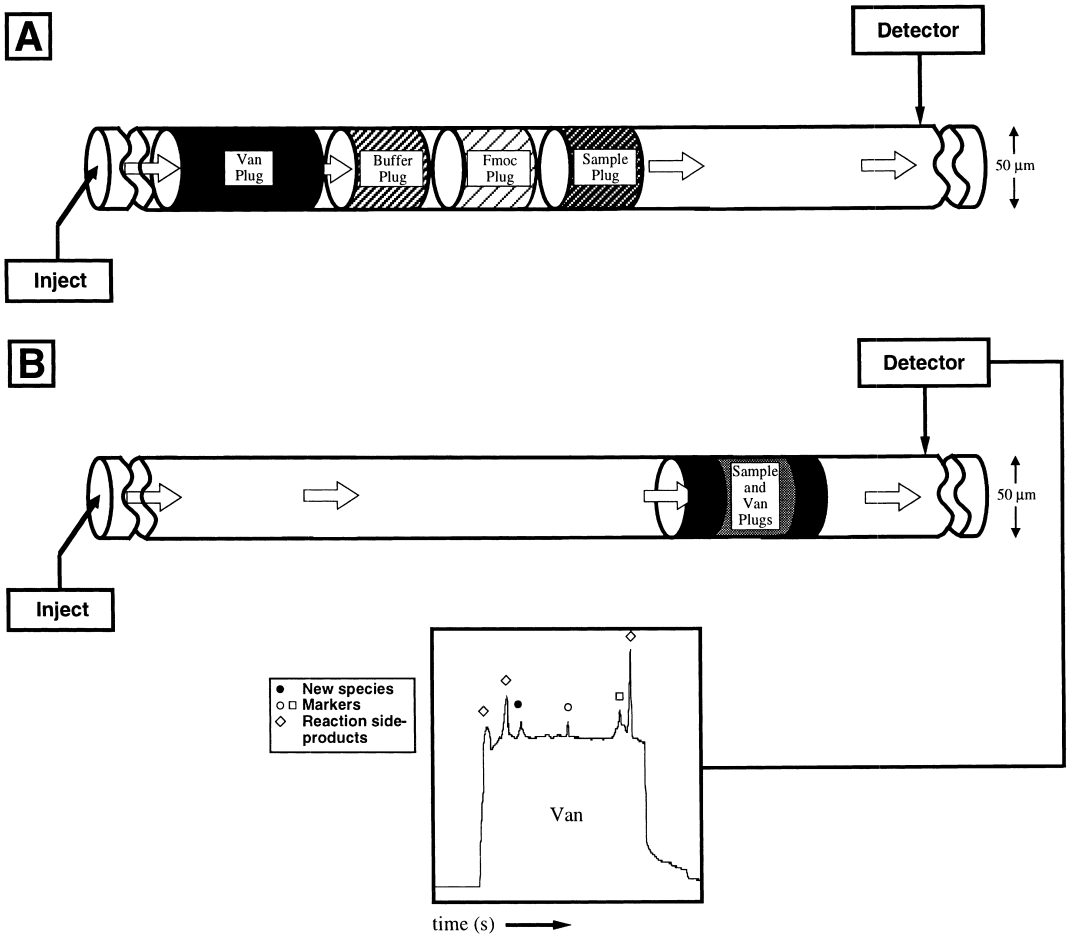


Fig. 2. Schematic of an on-column synthesis PFACE experiment.

2. Experimental

2.1. Chemicals and reagents

All chemicals were analytical grade. Fmoc-Gly–NHS ester, Fmoc-Ala–NHS, Gly–Ala–Ala–D-Ala–D-Ala, and D-Ala–D-Ala–D-Ala–D-Ala were purchased from Bachem California (Torrance, CA, USA) and used without further purification. 4-Carboxybenzenesulfonamide (CBSA) was purchased from Aldrich (Milwaukee, WI, USA) D-Ala–D-Ala, D-Ala–D-Ala–D-Ala, nicotinamide adenine dinucleotide (NAD), and Van were purchased from Sigma (St. Louis, MO, USA). Stock solution of Van (200 μmol/l), Gly–Ala–Ala–D-Ala–D-Ala (0.01 mol/l), D-Ala–D-Ala–D-Ala–D-Ala (0.01 mol/l), D-Ala–D-Ala (0.01 mol/l), D-Ala–D-Ala–D-Ala (0.01 mol/l), and 4-carboxybenzenesulfonamide (3 m*M*) were each prepared by dissolving in buffer (20 m*M* phosphate buffer; pH 7.5).

2.2. Apparatus

The CE system used in this study was a Beckman Model P/ACE 5510 (Fullerton, CA, USA). The capillary tubing (Polymicro Technologies, Phoenix, AZ, USA) was of 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. Data were collected and analyzed with Beckman System Gold software. The conditions used in CE were as follows: voltage, 24 kV; current, 35.4 μA; detection, 205 nm; temperature, 25±0.2°C.

2.3. Procedures

A sample solution (1.2 nl) (a 1-s time of injection equates to 1.2 nl of volume of liquid), containing D-Ala–D-Ala terminus peptides, NAD and CBSA were introduced by pressure injection onto the capillary equilibrated with buffer (20 m*M* phosphate; pH 7.5). Separate plugs (2.4 nl each) of Fmoc-amino acid–NHS ester in acetonitrile and buffer (20 m*M* phosphate; pH 7.5) were next introduced by pressure injection and electrophoresed. In the binding studies a solution of Van (about 491 nl) at increasing concentration (0~80 μmol/l) was introduced after the buffer plug by voltage injection (24 kV, 1.5 min) and the electrophoresis run at 24 kV to complete detection of all species.

3. Results and discussion

In our first series of experiments we explored the efficacy of synthesizing peptidyl ligands using an on-column CE approach. Peptidyl ligands having a D-Ala–D-Ala terminus bind to Van at affinities in the micromolar range. One route to these species involves the reaction of an Fmoc-amino acid–NHS ester and a D-Ala–D-Ala terminus peptide forming the corresponding Fmoc-amino acid–D-Ala–D-Ala ligand. A number of derivatized D-Ala–D-Ala species containing a variety of N-terminus moieties have been synthesized.

Our initial work focussed on optimizing the on-column microreaction procedure using the peptides D-Ala–D-Ala, D-Ala–D-Ala–D-Ala, D-Ala–D-Ala–D-Ala–D-Ala, and Gly–Ala–Ala–D-Ala–D-Ala as our test set of ligand precursors. In this control experiment a plug of sample containing the four test peptides and two non-interacting markers, NAD and CBSA, dissolved in phosphate buffer, was injected onto the capillary column. The column was subsequently partially-filled with a solution of Fmoc-Gly–NHS ester dissolved in acetonitrile and electrophoresed. Fmoc-Gly–NHS ester is neutral at pH 7.5 and elutes with electroosmotic flow (EOF). The test set of peptides are slightly negatively charged at this pH and migrate through the column at a lower velocity than the Fmoc-Gly–NHS ester, thereby facilitating overlap of the two zones upon electrophoresis forming four new Fmoc-derivatized peptide species, Fmoc-Gly–D-Ala–D-Ala, **1**, Fmoc-Gly–D-Ala–D-Ala–D-Ala, **2**, Fmoc-Gly–D-Ala–D-Ala–D-Ala–D-Ala, **3**, and Fmoc-Gly–Gly–Ala–Ala–D-Ala–D-Ala, **4**. Differences in transient velocities cause the faster migrating plug to penetrate the zone of the slower migrating plug under an applied field. The order of injection of the materials are dependent on the electrophoretic mobilities of the samples.

Fig. 3 is a representative electropherogram showing ligands **1–4** synthesized using an on-column synthesis procedure at 205 nm. As can be seen the migration times of the peptides elute at greater migration times as the size of the peptide decreases

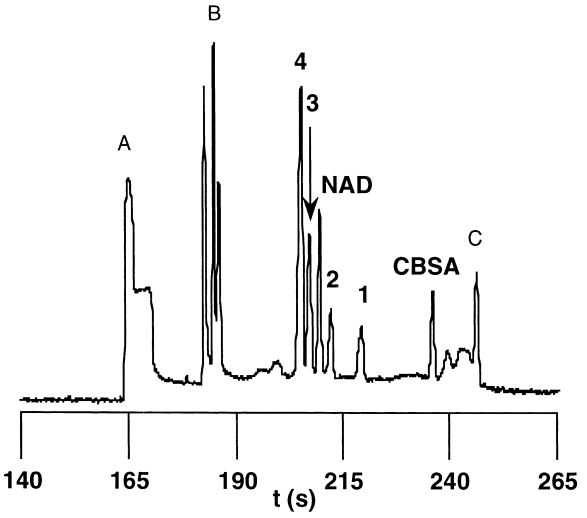


Fig. 3. Electropherogram of Fmoc-Gly–D-Ala–D-Ala, **1**, Fmoc-Gly–D-Ala–D-Ala–D-Ala, **2**, Fmoc-Gly–D-Ala–D-Ala–D-Ala–D-Ala, **3**, and Fmoc-Gly–Gly–Ala–Ala–D-Ala–D-Ala, **4**, in 20 m*M* phosphate buffer (pH 7.5) at 205 nm. The total analysis time in each experiment was 5.0 min at 24 kV (current: 35.4 μA) using a 40.5 cm (inlet to detector) 50 μm I.D. open, uncoated quartz capillary. NAD and CBSA were used as internal standards. A–C are explained in the text.

in molecular mass. Peaks A–C are unreacted Fmoc-Gly–NHS ester (A), unreacted Gly–D-Ala–D-Ala, Gly–D-Ala–D-Ala–D-Ala, Gly–Ala–Ala–D-Ala–D-Ala, and Gly–Gly–Ala–Ala–D-Ala–D-Ala (B), and Fmoc-Gly (C). Peaks were assigned in the electropherogram based on preliminary experiments using ligands synthesized off-column.

In our second series of experiments we examined the on-column synthesis of a single D-Ala–D-Ala terminus peptide and its binding to Van using an on-column synthesis PFACE technique. Here, plugs of D-Ala–D-Ala–D-Ala–D-Ala and two non-interacting markers dissolved in phosphate buffer, Fmoc-Ala–NHS ester dissolved in acetonitrile, buffer, and Van in phosphate buffer partially-filled into the capillary column, are injected and electrophoresed. NAD and CBSA were used as markers in the analysis and do not interact with any of the compounds in the running buffer under conditions of electrophoresis. Their migration times remain constant during the course of the binding experiment. Overlap of the separate zones of species yields the new Fmoc-Ala–D-Ala–D-Ala–D-Ala–D-Ala, **5**, species. The zone of Van then migrates into the zone of **5** and a dynamic equilibrium is achieved between Van and **5**. Fmoc-Ala–NHS ester is dissolved in acetonitrile because it is readily hydrolyzed in aqueous solution which would hinder the formation of **5**. The plug of buffer is intended to prevent early overlap of the Van and reaction plugs prior to synthesis of the new Fmoc species.

Fig. 4 shows a representative series of electropherograms of **5** in a capillary partially-filled with

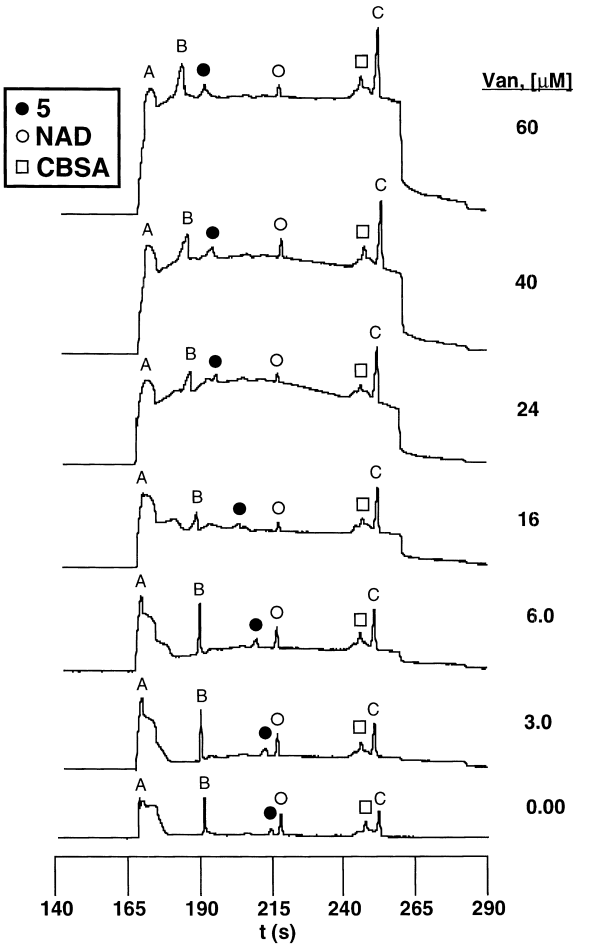


Fig. 4. A representative series of electropherograms of Fmoc-Ala–D-Ala–Ala–D-Ala–D-Ala, **5**, in 20 m*M* phosphate buffer (pH 7.5) at 205 nm containing various concentrations of Van using the on-column synthesis PFACE technique. The total analysis time in each experiment was 5.0 min at 24 kV (current: 35.4 μA) using a 40.5 cm (inlet to detector) 50 μm I.D. open, uncoated quartz capillary.

increasing concentrations of Van at 205 nm. Addition of increasing concentrations of Van in the running buffer shifts **5** to the left (shorter migration time) for all concentrations of Van. The peak for **5** shifts to the left because the mass of the newly formed complex is greater than the ligand itself. At the highest concentration of Van a 25 s shift in **5** is observed between uncomplexed and complexed forms. In these experiments approximately 16.8 pmol of peptide and 74 pmol of Van are used in the binding assay. The height of the Van plateaus in Fig. 4 increase due to the increased concentration of Van partially-filled in the capillary column. Three other peaks are observed in the electropherograms and are identified as unreacted Fmoc–Ala–NHS ester (A), D-Ala–D-Ala–D-Ala–D-Ala (B), and Fmoc-Ala acid (C). These species do not interfere in the PFACE binding studies. Based on the peak height of A, B, and C, we estimate the yield of **5** to be approximately 15%. It is apparent for the series of electropherograms that D-Ala–D-Ala–D-Ala–D-Ala has some affinity for Van since a shift to shorter migration times is observed. Unfortunately, we were unable to elucidate a binding constant for this interaction. It is also evident from the series of electropherograms that the conversion of D-Ala–D-Ala–D-Ala–D-Ala to **5** is constant throughout the duration of the experiment. Analysis of the data at wavelengths other than 205 nm was possible but resulted in reduced peak heights for both **5** and the non-interacting markers making PFACE analysis difficult.

Fig. 5 is a Scatchard plot of the data for **5** using the relative migration time ratio, RMTR, as the basis for the analysis [21]. In experiments employing two non-interacting standards, K_b can be estimated by relating the two standards to the species being studied using the RMTR (Eq. (1)).

$$\mathrm{RMTR} = (t_r - t_s')/(t_s' - t_s) \quad (1)$$

Here, t_r, t_s, and t_s' are the measured migration times of the peptide ligand and the two non-interacting standards, respectively. A Scatchard plot can be obtained using Eq. (2).

$$\Delta\mathrm{RMTR}_{R,L}/[L] = K_b\Delta\mathrm{RMTR}_{R,L}^{\max} - K_b\Delta\mathrm{RMTR}_{R,L} \quad (2)$$

Here, $\Delta\mathrm{RMTR}_{R,L}$ is the magnitude of the change in the RMTR as a function of the concentration of ligand. Eq. (2) allows for the estimation of K_b on a relative time scale using two non-interacting standards and compensates for fluctuations in the capillary column induced by electrophoresis. In the present experiment, t_s and t_s' are the migration times of NAD and CBSA, respectively. Using the RMTR a binding constant of $22.6\cdot10^3$ M^{-1} is obtained for the interaction of **5** and Van.

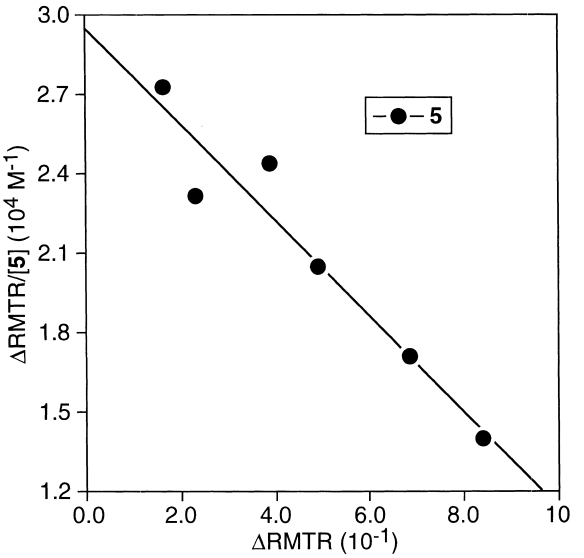


Fig. 5. Scatchard plot of the data for **5** according to Eq. (2).

To assess the correctness of the on-column ligand synthesis PFACE technique we conducted a separate ACE experiment between **5** and Van. Compound **5** was initially synthesized off-column and used for the ACE studies. A sample of **5**, NAD, and CBSA were injected onto the column and electrophoresed to obtain the RMTR for **5**. Increasing concentrations (0–80 μM) of Van were subsequently injected onto the column thereby inducing changes in the migration time of **5**. Measurement of the RMTR due to complexation of **5** and Van resulted in a binding constant of $17.7\cdot10^3$ M^{-1}. This value is slightly smaller than that obtained using the on-column ligand synthesis PFACE technique. A factor that may have contributed to the observed differences in binding constants between the on-column PFACE and standard ACE techniques is the small variation in Fmoc species formed between runs of the former

technique. If too great a deviation in the amount of peptide formed on-column occurs such variations may influence the ratio of bound to unbound peptide and, hence, the migration time of the peptide. Still, we believe that since multiple electrophoresis runs were used for the Scatchard analysis such deviations caused by variable synthetic yields are minimized over the totality of the analysis.

In a final series of experiments we examined the on-column synthesis of multiple Fmoc-derivatized peptides and their respective analysis by PFACE. Here, a plug of sample containing Ala–D-Ala–D-Ala, D-Ala–D-Ala–D-Ala–D-Ala, and Gly–Ala–Ala–D-Ala–D-Ala, NAD and CBSA, was injected into the capillary followed by separate plugs of Fmoc-Gly–NHS ester and buffer. The capillary was subsequently partially-filled with increasing concentrations of Van and electrophoresed. The overlap of the first two zones of material allow for the formation of three different Fmoc-derivatized species, Fmoc-Gly–D-Ala–D-Ala–D-Ala, **2**, Fmoc-Gly–D-Ala–D-Ala–D-Ala–D-Ala, **3**, and Fmoc-Gly–Gly–Ala–Ala–D-Ala–D-Ala, **4**, each eluting at distinct migration times. Fig. 6 shows a representative series of electropherograms of peptides **2–4** in buffer containing increasing concentrations of Van. All three Fmoc species elute at earlier migration times than in the absence of Van in the running buffer. Peaks A–C are identified as unreacted Fmoc-Gly–NHS ester (A), D-Ala–D-Ala–D-Ala, D-Ala–D-Ala–D-Ala–D-Ala, and Gly–Ala–Ala–D-Ala–D-Ala (B), and Fmoc-Gly-acid (C).

Fig. 7 is a Scatchard plot of the data for **2–4** using the RMTR as the form of analysis. Overall, eight different D-Ala–D-Ala terminus peptides were examined the results of which are summarized in Table 1. To our knowledge binding constants for ligands **3–8** have yet been reported. The average correlation coefficients (R) for the eight ligands are 0.9599 and 0.9572 for the on-column synthesis PFACE and traditional ACE techniques, respectively. This demonstrates that the on-column synthesis PFACE technique is a viable method to measure binding constants.

For the on-column synthesis PFACE technique to be accepted and generally used several criteria must first be satisfied. One, the reaction must be kinetically favorable since the length of time both substrate and derivatization reagent are in contact may

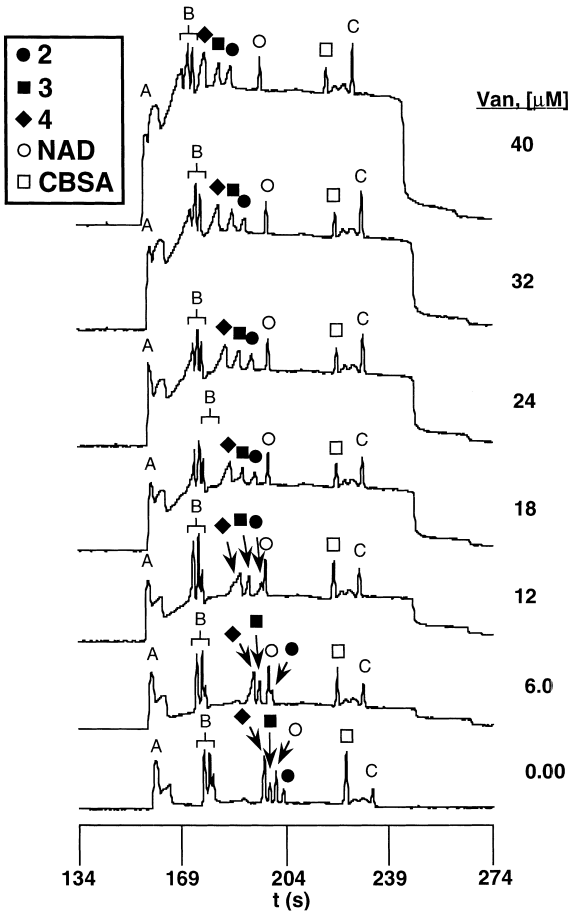


Fig. 6. A representative series of electropherograms of Fmoc-Gly–D-Ala–D-Ala–D-Ala, **2**, Fmoc-Gly–D-Ala–D-Ala–D-Ala–D-Ala, **3**, and Fmoc-Gly–Gly–Ala–Ala–D-Ala–D-Ala, **4**, in 20 mM phosphate buffer (pH 7.5) at 205 nm containing various concentrations of Van using the on-column synthesis PFACE technique. The total analysis time in each experiment was 5.0 min at 24 kV (current: 35.4 μA) using a 40.5 cm (inlet to detector) 50 μm I.D. open, uncoated quartz capillary. NAD and CBSA were used as internal standards. A–C are explained in the text.

be only a few seconds. Two, the electrophoretic mobilities of ligand and receptor must be different in order for the zones to overlap upon electrophoresis. Three, knowledge about the electrophoretic mobilities of the receptor, non-interacting standards, and ligand is necessary in order to determine if overlap is sufficient at the point of detection. Four, a sufficient amount of ligand must be injected onto the capillary to ensure a dynamic equilibrium is achieved between it and the receptor. Five, receptor, non-interacting

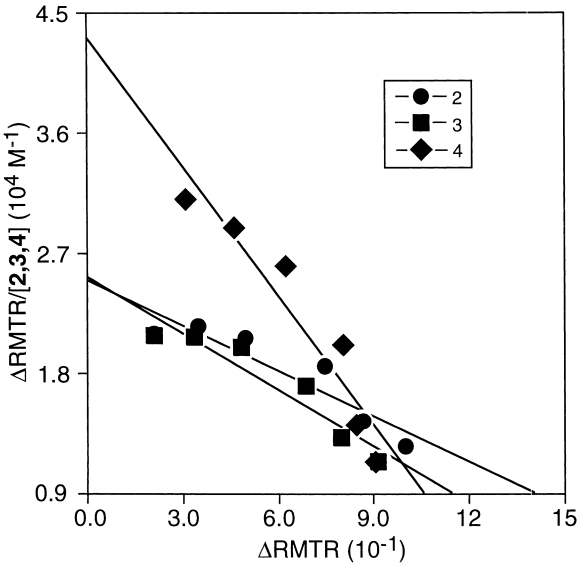


Fig. 7. Scatchard plot of the data for **2–4** according to Eq. (2).

markers, and ligand(s) must not absorb onto the walls of the capillary.

4. Conclusion

On-column ligand synthesis coupled to PFACE can effectively be used to estimate binding constants of D-Ala–D-Ala peptides to Van. In these experiments the capillary is sequentially filled with peptide, Fmoc-derivatizing reagent, buffer, and Van in increasing concentrations, and electrophoresed. Binding constants are estimated using the relative migration time ratio. The binding constants obtained by this technique are in agreement with those values obtained by traditional assay methods and by other ACE techniques. On-column ligand synthesis coupled to PFACE has several advantages as a method for measuring biomolecular non-covalent interactions. First, it requires smaller quantities of both receptor and ligand than in traditional ACE techniques. Second, purified ligand and/or receptor are not always required as long as the peak of interest can be differentiated from other peaks. Third, the technique can be modified for high-throughput analysis of bimolecular non-covalent interactions if the reaction part of the technique affords products on the timescale of CE. Fourth, the commercial availability of automated instrumentation, and the high reproducibility of data, make it experimentally convenient. Further work to demonstrate the versatility of this two-step technique is in progress.

Table 1
Experimental values of binding constants K_b (10^3 M^{-1}) of ligands **1–8** and vancomycin measured by the on-column synthesis PFACE technique

Ligand	Peptide	K_b [a]	K_b [b]
1	Fmoc-Gly–D-Ala–D-Ala	41.6[c]	[d]
2	Fmoc-Gly–D-Ala–D-Ala–D-Ala	14.9[e]	[d]
3	Fmoc-Gly–D-Ala–D-Ala–D-Ala–D-Ala	15.1	16.1
4	Fmoc-Gly–Gly–Ala–Ala–D-Ala–D-Ala	31.0	25.5
5	Fmoc-Ala–D-Ala–D-Ala–D-Ala–D-Ala–D-Ala	22.6	17.7
6	Fmoc-Ala–D-Ala–D-Ala	174.5	109.8
7	Fmoc-Ala–D-Ala–D-Ala–D-Ala	34.3	33.7
8	Fmoc-Ala–Gly–Ala–Ala–D-Ala–D-Ala	36.7	24.2

[a] The reported binding constants are the average values from two or three experiments for ligands **1–8**.
[b] Binding constants obtained using a standard ACE technique.
[c] Previous estimate [28]: $K_b = 19.0 \cdot 10^3$ M^{-1}.
[d] Not estimated in this study.
[e] Previous estimate [28]: $K_b = 14.0 \cdot 10^3$ and $30 \cdot 10^3$ M^{-1}.

Acknowledgements

The authors gratefully acknowledge financial support for this research by grants from the National Science Foundation CAREER (CHE-9703142), CRUI Programs (DBI-9710796), and National Institutes of Health Minority Biomedical Research Support (MBRS) Program (GM61331).

References

[1] S.C. Beale, Anal. Chem. 70 (1998) 279R.
[2] S.A. Zugel, B.J. Burke, F.E. Regnier, F.E. Lytle, Anal. Chem. 72 (2000) 5731.
[3] Z. Jin, R. Chen, L.A. Colon, Anal. Chem. 69 (1997) 1326.
[4] E.-S. Kwak, S. Esquivel, F.A. Gomez, Anal. Chim. Acta 397 (1999) 183.
[5] N. Chiem, D.J. Harrison, Anal. Chem. 69 (1997) 373.
[6] D.H. Patterson, B.J. Harmon, F.E. Regnier, J. Chromatogr. A 732 (1996) 119.
[7] W. Nashabeh, Z. El Rassi, J. Chromatogr. 596 (1992) 251.
[8] L.Z. Avila, G.M. Whitesides, J. Org. Chem. 58 (1993) 5508.
[9] Q. Xue, E.S. Yeung, Anal. Chem. 66 (1994) 1175.
[10] L.A. Amankwa, W.G. Kuhr, Anal. Chem. 64 (1992) 1610.

[11] D.H. Patterson, B.J. Harmon, F.E. Regnier, J. Chromatogr. A 662 (1994) 389.
[12] L.J. Cole, R.T. Kennedy, Electrophoresis 16 (1995) 549.
[13] L.B. Koutny, D. Schmalzing, T.A. Taylor, Anal. Chem. 68 (1996) 18.
[14] J. Gao, M. Mammen, G.M. Whitesides, Science 272 (1996) 535.
[15] X.-H. Qian, K.B. Tomer, Electrophoresis 19 (1998) 415.
[16] Y.-H. Chu, Y.M. Dunayevskiy, D.P. Kirby, P. Vouros, B.L. Karger, J. Am. Chem. Soc. 118 (1996) 7827.
[17] Y.M. Dunayevskiy, Y.V. Lyubarskaya, Y.-H. Chu, P. Vouros, B.L. Karger, J. Med. Chem. 41 (1998) 1201.
[18] Y.-H. Chu, L.Z. Avila, H.A. Biebuyck, G.M. Whitesides, J. Org. Chem. 58 (1993) 648.
[19] Y.-H. Chu, D.P. Kirby, B.L. Karger, J. Am. Chem. Soc. 117 (1995) 5419.
[20] F.A. Gomez, J.N. Mirkovich, V.M. Dominguez, K.W. Liu, D.M. Macias, J. Chromatogr. A 727 (1996) 291.
[21] E. Mito, Y. Zhang, S. Esquivel, F.A. Gomez, Anal. Biochem. 280 (2000) 209.
[22] M. Fang, L. Sheng, L.H. Han, X. Yu, R. Zhao, B. Zang, G. Liu, Electrophoresis 20 (1999) 1846.
[23] V.A. VanderNoot, R.E. Hileman, J.S. Dordick, R.J. Linhardt, Electrophoresis 19 (1998) 437.
[24] J.J. Colton, J.D. Carbeck, J. Rao, G.M. Whitesides, Electrophoresis 19 (1998) 367.
[25] M.H.A. Busch, L.B. Carels, H.F.M. Boelens, J.C. Kraak, H. Poppe, J. Chromatogr. A 777 (1997) 311.
[26] G.-H. Wan, X.C. Le, Anal. Chem. 72 (2000) 5583.
[27] F.A. Gomez, L.Z. Avila, Y.-H. Chu, G.M. Whitesides, Anal. Chem. 66 (1994) 1785.
[28] Y.-H. Chu, G.M. Whitesides, J. Org. Chem. 57 (1992) 3524.
[29] S. Handwerger, M. Pucci, K.J. Volk, J. Liu, M.S. Lee, J. Bacteriol. 176 (1994) 260.
[30] F. Lynen, Y. Zhao, Ch. Becu, F. Borremans, P. Sandra, Electrophoresis 20 (1999) 2462.
[31] E.-S. Kwak, F.A. Gomez, Chromatographia 43 (1996) 659.
[32] F.B. Erim, J.C. Kraak, J. Chromatogr. B 710 (1998) 205.
[33] E. Mito, F.A. Gomez, Chromatographia 50 (1999) 689.
[34] J. Heintz, M. Hernandez, F.A. Gomez, J. Chromatogr. A 840 (1999) 261.
[35] D. Amini, D. Westerlund, Anal. Chem. 70 (1998) 1425.
[36] T. Tanaka, S. Terabe, Chromatographia 44 (1997) 119.
[37] M. Ge, Z. Chen, H.R. Onishi, J. Kohler, L.L. Silver, R. Kerns, S. Fukuzawa, C. Thompson, D. Kahne, Science 284 (1999) 507.
[38] P.H. Popeniak, R.G. Pratt, Anal. Biochem. 165 (1987) 108.
[39] M. Nieto, H.R. Perkins, Biochem. J. 123 (1971) 789.
[40] T.D.H. Bugg, G.D. Wright, S. Dutka-Malen, M. Arthur, P. Courvalin, C.T. Walsh, Biochemistry 30 (1991) 10408.
[41] Y. Zhang, X. Gong, H. Zhang, R.C. Larock, E.S. Yeung, J. Comb. Chem. 2 (2000) 450.
[42] S.X. Sun, J. Hendrick, T. Staller, M. Sepaniak, J. Microcol. Sep. 10 (1998) 653.
[43] C.Z. Li, L.M. Martin, Anal. Biochem. 263 (1998) 72.