

Estimation of Receptor–Ligand Interactions by the Use of a Two-Marker System in Affinity Capillary Electrophoresis

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The study of receptor–ligand interactions by affinity capillary electrophoresis (ACE) requires an accurate form of analysis. Here, we examine the use of two noninteracting standards (markers) in the analysis of binding constant data in ACE studies. This concept is demonstrated using two model systems: carbonic anhydrase B (CAB, EC 4.2.1.1) and arylsulfonamides, and vancomycin (Van) from *Streptomyces orientalis* and the dipeptide *N*-acetyl-D-Ala-D-Ala. In this procedure a plug of receptor and noninteracting standards is injected, and analysis of the change in the relative migration time ratio of the receptor, relative to the noninteracting standards, as a function of the concentration of the ligand yields a value for the binding constant. The findings described here demonstrate that data from ACE studies can best be analyzed using two noninteracting standards, yielding values comparable to those estimated using other binding and ACE techniques. © 2000 Academic Press

Key Words: affinity capillary electrophoresis; relative migration time ratio; multiple-plug binding assay; flow-through partial-filling affinity capillary electrophoresis.

The characterization of molecular affinity coupled to the structural and quantitative information about the interacting molecules is necessary to understand the functions and mechanisms of biological systems in health and disease. The powerful combination of separation and molecular characterization that is possible by affinity capillary electrophoresis (ACE)² has made it

possible to study many interactions and to estimate binding parameters (1–38). The technique uses the resolving power of capillary electrophoresis to distinguish between free and bound forms of a receptor as a function of the concentration of free ligand (1). A number of interactions have been examined by ACE, affording information on binding parameters. For example, Heintz *et al.* have demonstrated the use of a partial-filling ACE technique in estimating binding constants of ligands to receptors (2). Dunayevskiy *et al.* have demonstrated that ACE can be used in combinatorial approaches to drug design by evaluating binding constants of a number of peptides to vancomycin (3). ACE has also been used to investigate an epitope on human immunodeficiency virus by a monoclonal antibody (4).

A number of forms of analysis have been utilized to estimate binding constants using ACE (5). Many of these forms require measurement of receptor electrophoretic mobilities in both complexed and uncomplexed states. Equation [1] is but one example of an equation based on electrophoretic mobilities that has been used successfully to estimate the K_b of ligands to receptors. In this equation changes in the electrophoretic mobility μ_R of a receptor (R) on complexation with a ligand (L) present in the buffer can be correlated to the binding constant K_b . Analysis of the magnitude of the change in mobility $\Delta\mu_{R,L}$ as a function of the concentration [L] of ligand yields K_b :

$$\Delta\mu_{R,L}/[L] = K_b\Delta\mu_{R,L}^{\max} - K_b\Delta\mu_{R,L} \quad [1]$$

Recently, mobility ratios, M , were used to estimate binding constants between ligands and receptors (6). Here, M is defined as $(t_{eo}/t_r) + 1$, where t_{eo} and t_r are

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² Abbreviations used: ACE, affinity capillary electrophoresis; RMT, relative migration time ratio; CAB, carbonic anhydrase B; Van, vancomycin; HHM, horse heart myoglobin; MO, mesityl oxide;

MPBA, multiple-plug binding assay; FTPFACE, flow-through partial-filling affinity capillary electrophoresis.

the migration times of a noninteracting standard and the receptor in question, respectively. Equation [2] is subsequently used for Scatchard analysis to estimate a K :

$$\Delta M_{R,L}/[L] = K_b \Delta M_{R,L}^{\max} - K_b \Delta M_{R,L}. \quad [2]$$

In practice, however, analyses dependent on electrophoretic mobilities are more susceptible to error than those independent of them due to fluctuations in electroosmotic flow (EOF). Although Eq. [2] has been most successful in estimating K_b compared to other forms of analyses, it is not yet a universal form of analysis and, hence, improvements in analysis of ACE data are still warranted.

In experiments employing two noninteracting standards, a relationship can be derived relating the standards to the receptor, which we term the relative migration time ratio, RMTR (Eq. [3]):

$$\text{RMTR} = (t_r - t'_s)/(t'_s - t_s). \quad [3]$$

Here, t_r , t_s , and t'_s are the measured migration times of the receptor peak and the two noninteracting standard peaks, respectively. A Scatchard plot can be obtained using Eq. [4]:

$$\Delta \text{RMTR}_{R,L}/[L] = K_b \Delta \text{RMTR}_{R,L}^{\max} - K_b \Delta \text{RMTR}_{R,L}. \quad [4]$$

Here, $\Delta \text{RMTR}_{R,L}$ is the magnitude of the change in the relative migration time ratio as a function of the concentration of ligand. Equation [4] allows for the estimation of K_b on a relative time scale using two noninteracting standards and compensates for fluctuations in the capillary column induced by electrophoresis.

In this paper we demonstrate the use of the relative migration time ratio to estimate binding constants of ligands to receptors in ACE. This concept is demonstrated using two model systems: carbonic anhydrase B (CAB, EC 4.2.1.1) and arylsulfonamides, and vancomycin (Van) from *Streptomyces orientalis* and the dipeptide *N*-acetyl-D-Ala-D-Ala. The general utility of this form of analysis is compared to other forms of analysis in ACE.

MATERIALS AND METHODS

Chemicals and reagents. All chemicals were analytical grade. 4-Carboxybenzenesulfonamide, **2** (Fig. 1), was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). *N*-Acetyl-D-Ala-D-Ala, **4**, vancomycin from *Streptomyces orientalis*, carbonic anhydrase B (CAB, EC 4.2.1.1, containing CAA and CAB isozymes, from bovine erythrocytes), and horse heart myoglobin (HHM) were purchased from Sigma Chemical Co. (St.

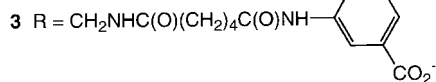
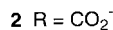
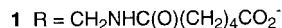
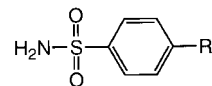


FIG. 1. Structures of compounds 1-3.

Louis, MO) and used without further purification. Mesityl oxide (MO) was purchased from Calbiochem (San Diego, CA). [[4-(Aminosulfonyl)phenyl]methylamino]-6-oxohexanoic acid, **1**, and [[[[4-(Aminosulfonyl)phenyl]methylamino]-1,6-dioxohexyl]amino]-1,3-benzenedicarboxylic acid, **3**, were synthesized based on literature procedures (7). Stock solutions of vancomycin (1 mg/ml), bovine carbonic anhydrase B (1 mg/ml), and horse heart myoglobin (1-4 mg/ml) were each prepared by dissolving the lyophilized protein in buffer (192 mM glycine-25 mM Tris, pH 8.3).

Apparatus. The capillary electrophoresis (CE) system used in this study was a Beckman Model P/ACE 5510 (Fullerton, CA). The capillary tubing (Polymicro Technologies, Phoenix, AZ) was of uncoated fused silica with an internal diameter of 50 μm , length from inlet to detector of 50.5 or 60.5 cm, and 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, 25-30 kV; current, 5.2-5.8 μA ; detection, 200 nm; temperature, 25 \pm 2°C.

Procedures. For the multiple-plug binding assay technique, a sample (3.6 nl) of solution containing 0.2 mg/ml of carbonic anhydrase B, 0.1 mg/ml of horse heart myoglobin, and 0.08 mg/ml of mesityl oxide in buffer was introduced into the capillary by vacuum injection. The electrophoresis was carried out using a Tris-glycine buffer and appropriate concentrations of the arylsulfonamide ligand (0-120 μM). For vancomycin, a sample (3.6 nl) of solution containing 0.14 mg/ml of vancomycin, 0.2 mg/ml of carbonic anhydrase B, and 0.17 mg/ml of mesityl oxide in buffer was introduced into the capillary by vacuum injection. The electrophoresis was carried out using a sodium phosphate buffer and appropriate concentrations of **4** (0-1150 μM). For the flow-through partial affinity capillary electrophoresis technique, a sample of arylsulfonamide was vacuum injected into the capillary for 0.1 min at high pressure followed by a sample (3.6 nl) of solution for 3 s containing 0.14 mg/ml of CAB, 0.14 mg/ml of HHM, and 0.08 mg/ml of MO in buffer. The electrophoresis was carried out using a Tris-glycine buffer and repeated at increasing concentrations of the aryl-

sulfonamide ligand (0–80 μM for **1**, 0–64 μM for **2**) for 7.0 min for **1** (6.5 min for **2**). For vancomycin, a sample of **4** was vacuum injected into the capillary for 0.10 min at high pressure followed by a sample (3.6 nl) of solution for 3 s containing 0.035 mg/ml of vancomycin, 0.14 mg/ml of CAB, 0.14 mg/ml of HHM, and 0.08 mg/ml of MO in buffer. The electrophoresis was carried out using a Tris-glycine buffer and increasing concentrations of **4** (0–1150 μM) for 5.0 min.

RESULTS AND DISCUSSION

To determine the efficacy of the relative migration time ratio, RMTR, we examined the data obtained from two types of ACE techniques: the multiple-plug binding assay (MPBA) and flow-through partial-filling affinity capillary electrophoresis (FTPFACE). The multiple-plug binding assay is a technique whereby multiple values of K_b can be obtained for a receptor-ligand interaction (8). In this technique multiple plugs of receptor and noninteracting standards are injected and are subjected to increasing concentrations of ligand in the running buffer. Changes in the migration time between complexed and uncomplexed receptor are used for the analysis. In our initial studies we examined the interaction of carbonic anhydrase (CAB) and arylsulfonamides. In these experiments, four separate plugs of sample, each containing CAB, mesityl oxide (MO), and horse heart myoglobin (HHM), were injected and electrophoresed. MO and HHM were used as the noninteracting standards. We have much experience with both MO and HHM and have found that neither species sticks to the capillary column at the pH of study nor degrades over time during the course of the ACE study. They also do not interact with either the receptor or ligand or cause perturbations in electroosmotic flow (EOF) vis-à-vis the running buffer. The concentration of **1** was successively increased from 0 to 120 μM .

Figure 2 shows a representative series of electropherograms of CAB in buffer containing various concentrations of **1**. Upon addition of increasing concentrations of **1** in the running buffer, the four CAB peaks shift to the right for any given concentration. CAB, when bound to **1**, is more negative than in the unbound state and was detected later than the uncomplexed form. Peak broadening was observed at intermediate concentrations and is caused by the retardation of migrating molecules due to their frequent interactions with the ligand in the region of intermediate status (8). The CAB peaks become sharper at the saturating concentrations of the ligand. The inverted peaks (*) resulted from the dilution of **1** present in the electrophoresis buffer. CAA (+), a protein having binding constants very similar to the binding constants of CAB, gives values of K_b that are indistinguishable from those of CAB.

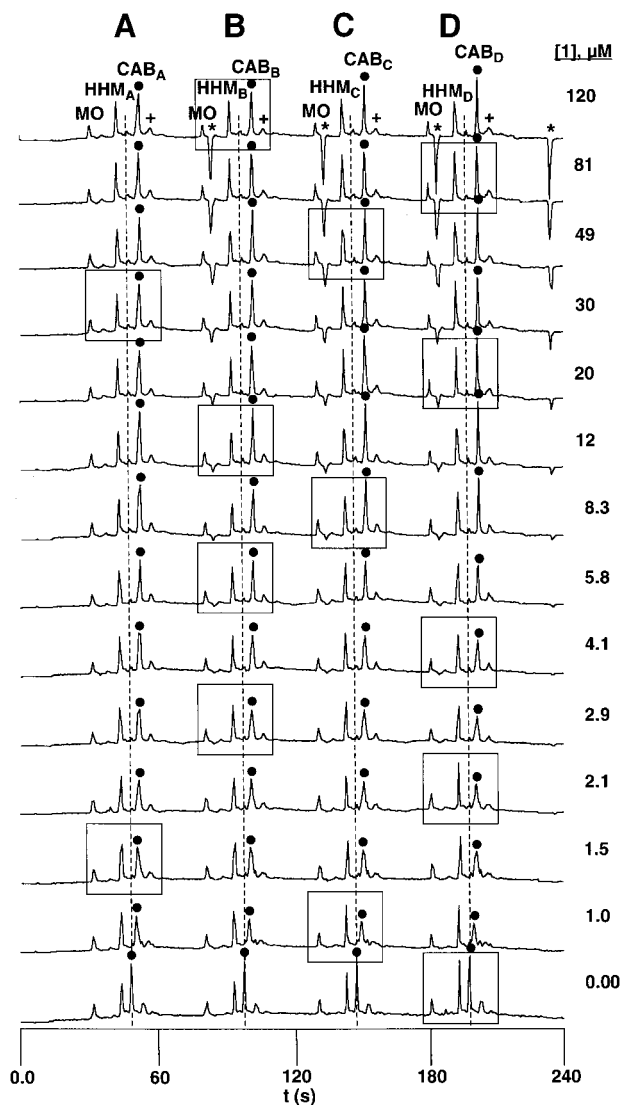


FIG. 2. A representative set of electropherograms of carbonic anhydrase B (CAB) in 0.192 M glycine–0.025 M Tris buffer (pH 8.3) containing various concentrations of **1**. The total analysis time in each experiment was 7.0 min at 30 kV (current: 5.2 μA) using a 60.5-cm (inlet to detector), 50- μm i.d. open, uncoated quartz capillary. Horse heart myoglobin (HHM) and mesityl oxide (MO) were used as internal standards. The inverted peaks (*) and the peaks denoted as (+) are discussed in the text.

We then chose to examine a random set of sample plugs for the analysis. Using Eq. [4], we found that the apparent K_b value for the interaction of **1** with CAB was $0.66 \times 10^6 \text{ M}^{-1}$, comparable to previous ACE studies and other binding assays (1, 2, 6–11). Table 1 lists the binding constants obtained using Eqs. [1], [2], and [4]. Figure 3 is a Scatchard plot of the data according to Eq. [4]. It is clear from Fig. 3 that the use of the RMTR is an appropriate form of analysis of ACE data. The use of two noninteracting standards and the conversion of detection time data

TABLE 1

Experimental Values of Binding Constants K_b (10^6 M^{-1}) of Ligands 1–3 and Carbonic Anhydrase B Obtained by Eqs. [1], [2], and [4] Using the Multiple-Plug Binding Assay Technique

Ligand	K_b (correlation coefficient r)		
	Eq. [1]	Eq. [2]	Eq. [4]
1	<i>a</i>	<i>a</i>	0.66 ^b (0.989)
2	<i>a</i>	<i>a</i>	1.13 ^c (0.973)
3	<i>a</i>	<i>a</i>	0.74 ^d (0.966)

^a Unable to be measured.

^b Previous estimates (2, 6, 7, 8, 17): $K_b = (0.45\text{--}0.92) \times 10^6 \text{ M}^{-1}$.

^c Previous estimates (6, 7, 8, 17): $K_b = (0.72\text{--}2.0) \times 10^6 \text{ M}^{-1}$.

^d Previous estimates (6, 7, 8, 17): $K_b = (0.50\text{--}0.84) \times 10^6 \text{ M}^{-1}$.

to relative migration time ratio change data according to Eq. [4] are effective in canceling out the contribution of EOF. In this experiment the two markers elute prior to the CAB peak but can still be used as markers in the RMTR form of analysis. Values for K_b cannot be estimated using Eqs. [1] and [2]. Figure 4 shows Scatchard plots of the data according to Eqs. [1] and [2]. These equations yield linear Scatchard plots only when the voltage and the capillary length are constant. Although both electrophoresis parameters are unchanged in the current set of experiments, the data have been randomly selected from different plugs of sample with varying times of injection, thereby simulating different capillary lengths. These changes are dramatic enough such that a Scatchard plot cannot be realized using equations based on changes in electrophoretic mobilities. Although Eq. [2] does not require measurement of electrophoretic mobilities, a binding constant cannot

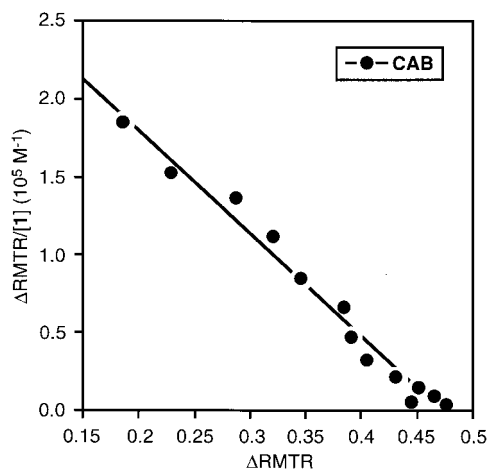


FIG. 3. Scatchard plot of the data for carbonic anhydrase B according to Eq. [4].

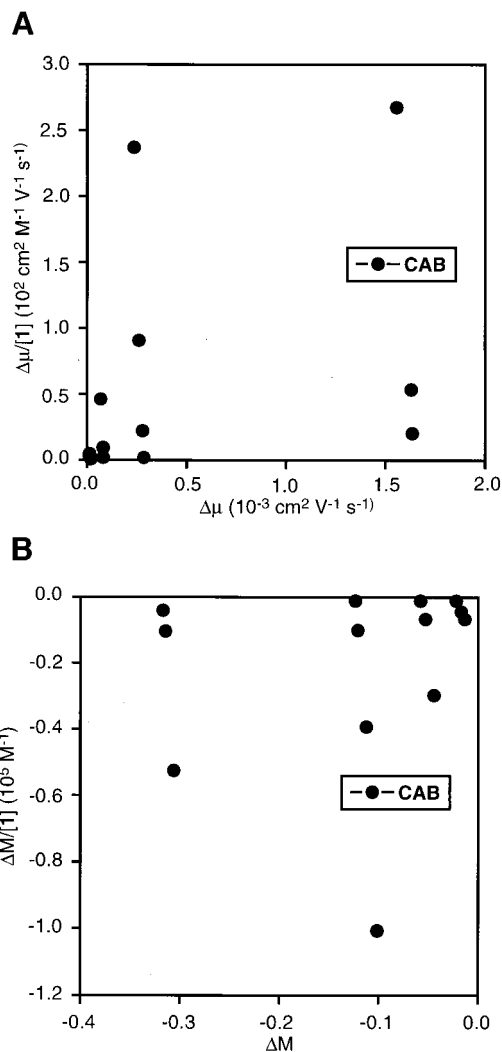


FIG. 4. Scatchard plots of the data for carbonic anhydrase B according to Eqs. (A) [1] and (B) [2].

be estimated using it as the basis of analysis. Equation [4] is independent of both capillary length and voltage and, hence, analysis of a random set of data still affords a value for K_b .

Similar experiments and analyses were conducted with vancomycin (Van) and the dipeptide *N*-acetyl-D-Ala-D-Ala. In these experiments four separate plugs of sample containing Van and MO and CAB, as noninteracting markers, were vacuum injected into the capillary and electrophoresed. Analysis by Eq. [4] yielded a binding constant of $4.5 \times 10^3 \text{ M}^{-1}$. Table 2 lists the binding constants obtained using Eqs. [1], [2], and [4]. Values for K_b cannot be estimated using Eq. [1] or [2]. The value for K_b using Eq. [4] is comparable to that obtained using conventional ACE experiments and by other assay techniques (6, 8). Unlike the CAB study, the two markers used in this study eluted both before and after the Van peak. Hence, the RMTR form of

TABLE 2

Experimental Values of Binding Constants K_b (10^3 M^{-1}) of Ligand **4** and Vancomycin Obtained by Eqs. [1], [2], and [4] Using the Multiple-Plug Binding Assay (MPBA) Flow-Through Partial-Filling Affinity Capillary Electrophoresis Technique (FTPFACE)

Technique	K_b (correlation coefficient r)		
	Eq. [1]	Eq. [2]	Eq. [4]
MPBA	^a	^a	4.5 ^b (0.946)
FTPFACE	3.1 ^b (0.947)	3.2 ^b (0.967)	3.7 ^b (0.959)

^a Unable to be measured.

^b Previous estimates (2, 6, 8, 9, 11): $K_b = (3.9-8.7) \times 10^3 \text{ M}^{-1}$.

analysis can be used when both markers elute both before the receptor peak and on either side of the receptor peak.

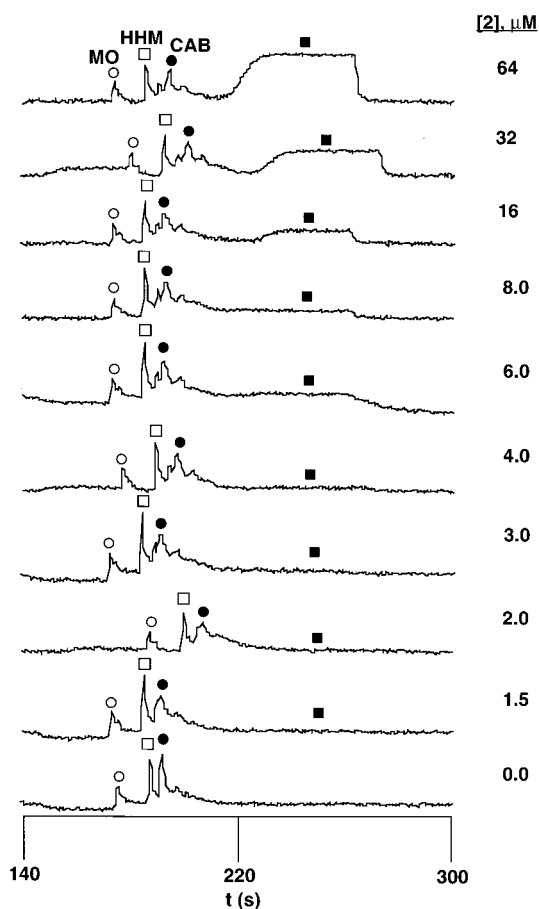


FIG. 5. A representative set of electropherograms of carbonic anhydrase B (CAB) in 0.192 M glycine-0.025 M Tris buffer (pH 8.3) containing various concentrations of **2** using the FTPFACE technique. The total analysis time in each experiment was 6.5 min at 28 kV (current: $5.8 \mu\text{A}$) using a 60.5-cm (inlet to detector), $50\text{-}\mu\text{m}$ i.d. open, uncoated quartz capillary. Mesityl oxide (MO) and horse heart myoglobin (HHM) were used as internal standards.

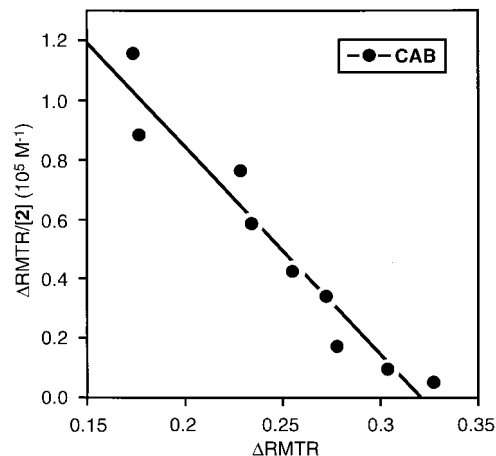


FIG. 6. Scatchard plot of the data for carbonic anhydrase B according to Eq. [4].

To further prove the versatility of the R form of analysis, we used the FTPFACE technique to examine receptor-ligand interactions (36, 38). In this technique a plug of ligand is initially injected followed by a small plug of sample containing receptor and two noninteracting standards. Upon electrophoresis the sample plug flows into the domain of the ligand plug, where a dynamic equilibrium between receptor and ligand is established. Continued electrophoresis enables the sample plug to flow through the ligand plug, where it is detected prior to the sample of ligand. Changes in the injection time between uncomplexed and complexed receptor are then used to estimate K_b . We initially examined the interaction between CAB and two arylsulfonamides. In this study a plug of **2** at increasing concentration was vacuum injected (0.1 min at high pressure) into the capillary at high pressure followed by a plug of sample (3 s at low pressure) containing CAB, HHM, and MO and electrophoresed for 6.5 min. HHM and MO are noninteracting standards used in the data analysis.

Figure 5 shows a representative series of electropherograms of CAB in capillaries partially filled with increasing concentrations (0-64 μM) of **2**. The complexation between **2** and CAB resulted in an increasing negative charge and the complex is detected later than the uncomplexed form. Figure 6 is a Scatchard plot of the data for CAB using Eq. [4]. Table 3 summarizes the binding data for the two ligands and CAB obtained by Eqs. [1], [2], and [4]. A stable EOF permits analysis of the data by all equations. The values obtained by Eq. [4] are in agreement with previous ACE studies on CAB and arylsulfonamides and with those obtained from other techniques (6, 8).

We also examined the interaction of vancomycin and the small peptide *N*-acetyl-D-Ala-D-Ala, **4**. In this study a plug of **4** at increasing concentrations was vacuum

TABLE 3

Experimental Values of Binding Constants K_b (10^6 M^{-1}) of Ligands **1** and **2** and Carbonic Anhydrase B Obtained by Eqs. [1], [2], and [4] Using the Flow-Through Partial-Filling Affinity Capillary Electrophoresis Technique

Ligand	K_b (correlation coefficient r)		
	Eq. [1]	Eq. [2]	Eq. [4]
1	0.59 (0.967)	0.84 (0.873)	0.57 ^a (0.970)
2	0.63 (0.869)	0.65 (0.933)	0.70 ^b (0.968)

^a Previous estimates (2, 6, 7, 8, 17): $K_b = (0.45\text{--}0.92) \times 10^6 \text{ M}^{-1}$.

^b Previous estimates (6, 7, 8, 17): $K_b = (0.72\text{--}2.0) \times 10^6 \text{ M}^{-1}$.

injected into the capillary for 0.10 min followed by a plug of sample containing Van, CAB, HHM, and MO and electrophoresed for 5.0 min. In the present experiment MO and HHM were used as the noninteracting standards and for the analysis and the subsequent Scatchard plot. The concentration of **4** was successively increased from 0 to 1150 μM . The values for the binding constants agree well with previous estimates by ACE and other binding techniques (6, 8).

The data presented herein demonstrate that binding constants using ACE can be estimated by the use of two noninteracting standards and the relative migration time ratio, RMTR. We have shown this by the use of two model systems: CAB and arylsulfonamide ligands, and vancomycin and the dipeptide *N*-acetyl-D-Ala-D-Ala. The binding constants obtained by this form of analysis agree well with those obtained by other assay techniques and other ACE forms of analysis. This form of analysis of ACE data has several advantages as a method for determining binding constants over other methods: (i) it does not require a stable EOF; (ii) data from multiple experiments conducted at varying times can be analyzed; and (iii) the analysis can be applied to a variety of ACE techniques.

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REFERENCES

1. Chu, Y.-H., Avila, L. Z., Gao, J., and Whitesides, G. M. (1995) Affinity capillary electrophoresis. *Acc. Chem. Res.* **28**, 461–468.
2. Heintz, J. M., Hernandez, M., and Gomez, F. A. (1999) Use of a partial-filling technique in affinity capillary electrophoresis for determining binding constants of ligand to receptors. *J. Chromatogr. A* **840**, 261–268.
3. Dunayevskiy, Y. M., Lyubarskaya, Y. V., Chu, Y.-H., Vouros, P., and Karger, B. L. (1998) Simultaneous measurement of nineteen binding constants of peptides to vancomycin using affinity cap-

- illary electrophoresis–mass spectrometry. *J. Med. Chem.* **41**, 1201–1204.
4. Qian, X.-H., and Tomer, K. B. (1998) Affinity capillary electrophoresis investigation of an epitope on human immunodeficiency virus recognized by a monoclonal antibody. *Electrophoresis* **19**, 415–419.
5. Rundlett, K. L., and Armstrong, D. W. (1997) Methods for the estimation of binding constants by capillary electrophoresis. *Electrophoresis* **18**, 2194–2202.
6. Kawaoka, J., and Gomez, F. A. (1998) Use of mobility ratios to estimate binding constants of ligands to proteins in affinity capillary electrophoresis. *J. Chromatogr. B* **715**, 203–210.
7. Gomez, F. A., Avila, L. Z., Chu, Y.-H., and Whitesides, G. M. (1994) Determination of binding constants of ligands to proteins by affinity capillary electrophoresis: Compensation for electroosmotic flow. *Anal. Chem.* **66**, 1785–1791.
8. Gomez, F. A., Mirkovich, J. N., Dominguez, V. M., Liu, K. W., and Macias, D. M. (1996) Multiple-plug binding assays using affinity capillary electrophoresis. *J. Chromatogr. A* **727**, 291–299.
9. Chu, Y.-H., and Whitesides, G. M. (1992) Affinity capillary electrophoresis can simultaneously measure binding constants of multiple peptides to vancomycin. *J. Org. Chem.* **57**, 3524–3525.
10. Zhao, D. S., Kwak, E.-S., Kawaoka, J., Esquivel, S., and Gomez, F. A. (1998) The use of affinity capillary electrophoresis for determining binding constants of ligands to receptors. *Am. Lab.* **30**, 40–47.
11. Chu, Y.-H., Avila, L. Z., Biebuyck, H. A., and Whitesides, G. M. (1992) Use of affinity capillary electrophoresis to measure binding constants of ligands to proteins. *J. Med. Chem.* **35**, 2915–2917.
12. Guszczynski, T., and Copeland, T. D. (1998) A binding shift assay for the zinc-bound and zinc-free HIV-1 nucleocapsid protein by capillary electrophoresis. *Anal. Biochem.* **260**, 212–217.
13. Sun, P., Hoops, A., and Hartwick, R. A. (1994) Enhanced albumin protein separations and protein–drug binding constant measurement using anti-inflammatory drugs as run buffer additives in affinity capillary electrophoresis. *J. Chromatogr. B* **661**, 335–340.
14. Ahmed, A., Ibrahim, H., Pastore, F., and Lloyd, D. K. (1996) Relationship between retention and effective selector concentration in affinity capillary electrophoresis and high-performance liquid chromatography. *Anal. Biochem.* **68**, 3270–3273.
15. Kuhn, R., Frei, R., and Christen, M. (1994) Use of capillary affinity electrophoresis for the determination of lectin–sugar interactions. *Anal. Biochem.* **218**, 131–135.
16. Erim, F. B., and Kraak, J. C. (1998) Vacancy affinity capillary electrophoresis to study competitive protein–drug binding. *J. Chromatogr. B* **710**, 205–210.
17. Colton, J. J., Carbeck, J. D., Rao, J., and Whitesides, G. M. (1998) Affinity capillary electrophoresis: A physical-organic tool for studying interactions in biomolecular recognition. *Electrophoresis* **19**, 367–382.
18. Shimura, K., and Karger, B. L. (1994) Affinity probe capillary electrophoresis: Analysis of recombinant human growth hormone with a fluorescently labeled antibody fragment. *Anal. Chem.* **66**, 9–15.
19. Lemesle-Lamache, V., Taverna, M., Wouessidjewe, D., Duchene, D., and Ferrier, D. (1993) Determination of the binding constant of salbutamol to unmodified and ethylated cyclodextrins by affinity capillary electrophoresis. *J. Chromatogr. A* **735**, 321–331.
20. Mammen, M., Colton, I. J., Carbeck, J. D., Bradley, R., and Whitesides, G. M. (1997) Representing primary electrophoretic

- data in the 1/time domain: Comparison to representations in the time domain. *Anal. Chem.* **69**, 2165–2170.
21. Heegaard, N. H. H., Nilsson, S., and Guzman, N. A. (1998) Affinity capillary electrophoresis: Important application areas and some recent developments. *J. Chromatogr. B* **715**, 29–54.
 22. Handwerger, S., Pucci, M., Volk, K. J., Liu, J., and Lee, M. S. (1994) Vancomycin-resistant *leuconostoc mesenteroides* and *lactobacillus casei* synthesize cytoplasmic peptidoglycan precursors that terminate in lactate. *J. Bacteriol.* **176**, 260–264.
 23. Liu, J., Volk, K. J., Lee, M. S., Pucci, M., and Handwerger, S. (1994) Binding studies of vancomycin to the cytoplasmic peptidoglycan precursors by affinity capillary electrophoresis *Anal. Chem.* **66**, 2412–2416.
 24. Carpenter, J. L., Camilleri, P., Dhanak, D., and Goodall, D. (1992) A study of the binding of vancomycin to dipeptides using capillary electrophoresis. *J. Chem. Soc., Chem. Commun.* 804–805.
 25. Busch, M. H. A., Kraak, J. C., and Poppe, H. (1997) Principles and limitations of methods available for the determination of association constants with affinity capillary electrophoresis. *J. Chromatogr. A* **777**, 329–353.
 26. Mammen, M., Gomez, F. A., and Whitesides, G. M. (1995) Determination of the binding of ligands containing the *N*-2,4-dinitrophenyl group to bivalent monoclonal rat anti-DNP antibody using affinity capillary electrophoresis. *Anal. Chem.* **67**, 3526–3535.
 27. Honda, S., Taga, A., Suzuki, K., Suzuki, S., and Kakehi, K. (1992) Determination of the association constant of monovalent mode protein–sugar interaction by capillary zone electrophoresis. *J. Chromatogr.* **597**, 377–382.
 28. Shimura, K., and Kasai, K. (1995) Determination of the affinity constants of concanavalin A for monosaccharides by fluorescence affinity probe capillary electrophoresis. *Anal. Biochem.* **227**, 186–194.
 29. Kwak, E.-S., and Gomez, F. A. (1996) Determination of the binding of β -cyclodextrin derivatives to adamantane carboxylic acids using capillary electrophoresis. *Chromatographia* **43**, 659–662.
 30. Vandernoot, V. A., Hileman, R. E., Dordick, J. S., and Linhardt, R. J. (1998) Affinity capillary electrophoresis employing immobilized glycosaminoglycan to resolve heparin-binding peptides. *Electrophoresis* **19**, 437–441.
 31. Chu, Y.-H., Dunayevskiy, Y. M., Kirby, D. P., Vouros, P., and Karger, B. L. (1996) Affinity capillary electrophoresis–mass spectrometry for screening combinatorial libraries *J. Am. Chem. Soc.* **118**, 7827–7835.
 32. Heegaard, N. H. H. (1998) A heparin-binding peptide from human serum amyloid P component characterized by affinity capillary electrophoresis. *Electrophoresis* **19**, 442–447.
 33. Busch, M. H. A., Carels, L. B., Boelens, H. F. M., Kraak, J. C., and Poppe, H. (1997) Comparison of five methods for the study of drug–protein binding in affinity capillary electrophoresis. *J. Chromatogr. A* **777**, 311–328.
 34. Lin, S., Hsiao, I.-Y., and Hsu, S.-M. (1997) Determination of the dissociation constant of phosphitin–anti-phosphoserine interaction by affinity capillary electrophoresis. *Anal. Biochem.* **254**, 9–17.
 35. Taga, A., Uegaki, K., Yabusako, Y., Kitano, A., and Honda, S. (1999) Simultaneous determination of the association constants of oligosaccharides to a lectin by capillary electrophoresis. *J. Chromatogr. A* **837**, 221–229.
 36. Tanaka, Y., and Terabe, S. (1997) Separation of the enantiomers of basic drugs by affinity capillary electrophoresis using a partial filling technique and an acid glycoprotein as chiral selector. *Chromatographia* **43**, 119–128.
 37. Valtcheva, L., Mohammad, J., Pettersson, G., and Hjerten, S. (1993) Chiral separation of beta-blockers by high-performance capillary electrophoresis based on non-immobilized cellulase as enantioselective protein. *J. Chromatogr. A* **638**, 263–267.
 38. Amini, A., and Westerlund, D. (1998) Evaluation of association constants between drug enantiomers and human α 1-acid glycoprotein by applying a partial-filling technique in affinity capillary electrophoresis. *Anal. Chem.* **70**, 1425–1430.