

Xiaojun Liu
Frank A. Gomez

Department of Chemistry and
Biochemistry, California State
University, Los Angeles, CA, USA

Received June 23, 2008
Revised July 28, 2008
Accepted August 2, 2008

Short Communication

Microchip frontal affinity chromatography to study the binding of a ligand to teicoplanin-derivatized microbeads

Microchip frontal affinity chromatography was demonstrated to estimate the binding of 5-carboxyfluorescein-(D-Ala)₃ (**1**) to magnetic microbeads derivatized with teicoplanin (Teic) from *Actinoplanes teicomyceticus*. In this technique, a cross-chip was used whereby the two side channels contained an identical length (1.5 mm) of derivatized Teic microbeads (affinity column) and underivatized beads (control column), respectively. Cylindrical NdFeB magnets were fabricated into the PDMS chips to retain the magnetic beads. Upon application of a voltage, a sample of **1** was continuously introduced into the affinity column followed by a buffer wash and the same sample from the control channel. The extent of interaction between **1** and the two types of beads in either microchannel resulted in differences in migration time of the ligand as detected by fluorescence. This difference was used to obtain a value for the binding constant between **1** and Teic-beads of $5.4 \times 10^4 \text{ M}^{-1}$. This technique reduces the amount of sample needed for the binding assay as compared with conventional frontal affinity chromatography techniques.

Keywords:

Binding constant / CE / Microchip frontal affinity chromatography

DOI 10.1002/elps.200800393

Since its inception 40 years ago [1], affinity chromatography (AC) has been widely applied to separate biochemical mixtures. More recently, AC has been used in chiral separations, sample preparation and clean-up, on-line proteolytic digestion, biocatalysis and for screening libraries of potential lead-compounds [2–7]. The latter application used AC in the frontal analysis mode (FAC) [5–7]. The basic premise of FAC is that a continuous infusion of a compound will allow for equilibration of the ligand between the free and bound states. FAC has several advantages. For example, the interaction in question can be easily measured without significantly altering the equilibrium. The experimental procedure is simple and the technique yields highly reproducible and accurate data [8]. Drawbacks of FAC include large sample consumption (usually mL quantities), low surface areas, high back pressures, difficulty in filling columns with beads, and adsorption of biomolecules onto the hydrophobic surface [9].

Over the past 10 years, MCE has become one of the most powerful analytical tools due to extremely low sample and reagent consumption, high separation

efficiency, miniaturization, high-throughput capabilities and easy integration [10–14]. MCE offers three modes for studying the binding of ligands to receptors: (i) microchip zone CE [15]; (ii) microchip ACE [16–18], and (iii) microchip frontal analysis [19–20]. Common to all three modes is that the receptor–ligand pair under investigation must alter the size, shape, and/or charge of one of the species to produce a sufficient change in the migration pattern. In microchip zone CE, the receptor, ligand, and complex should all have different electrophoretic mobilities to affect separation. In microchip ACE, the electrophoretic mobility of the free receptor must differ from the mobility of the complex and the kinetics of binding needs to be very fast. In microchip frontal analysis, there are two requirements for its use: (i) the mobilities of the receptor and complex should be similar, and (ii) the mobilities of the ligand and complex should be different.

Herein, we describe an electrokinetically controlled microfluidic system containing two magnetic microbead columns fabricated into the microchannels to estimate the binding of 5-carboxyfluorescein-(D-Ala)₃ (**1**) to magnetic microbeads derivatized with teicoplanin (Teic). One column is filled with Teic-modified beads and is used as the affinity column. The second column contains non-derivatized beads and is used as the control. The extent of interaction between **1** and the two types of beads in either microchannel resulted in differences in migration time of the ligand, and this is used in the Scatchard analysis to obtain a value for the binding constant.

The binding study was performed on a homemade microfluidic system consisting of two high voltage power

Correspondence: Dr. Frank A. Gomez, Department of Chemistry and Biochemistry, California State University, Los Angeles, 5151 State University Drive, Los Angeles, CA 90032-8202, USA
E-mail: fgomez2@calstatela.edu
Fax: +1-323-343-6490

Abbreviations: AC, affinity chromatography; FAC, frontal analysis mode; MFAC, microchip frontal affinity chromatography; Teic, teicoplanin

supplies and a confocal LIF detector described previously [21]. A three-step procedure was used to fabricate the microchips for microchip frontal AC (MFAC): (i) magnetic microbead derivatization with Teic; (ii) fabrication of a hydrophilic microchip; and (iii) packing of magnetic beads into the microchannels. A solution (100 μ L) of carboxylic acid-terminated magnetic beads from a stock solution (2×10^9 beads/mL) was washed ($5 \times$) with MES buffer (25 mM, pH 5). A solution (60 μ L) of Teic (10 mg/mL) in MES (25 mM, pH 5) was added to the beads and mixed. A solution (30 μ L) of freshly prepared N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) solution (100 mg/mL in cold water) and MES buffer (10 μ L) were added to the bead solution and the mixture was incubated for 6 h at room temperature with slight shaking. The solution was then discarded and beads were suspended in Tris (50 mM, pH 7.4) buffer for 15 min in order to quench non-reacted carboxylic acid groups. The beads were then washed with PBS (100 mM, pH 7.4, 150 mM NaCl, 0.1% BSA) ($5 \times$) and re-suspended in PBS (1 mL) (100 mM, pH 7.4, 150 mM NaCl) buffer.

The microchip was prepared by conventional soft photolithography techniques [10]. The cross pattern consisted of 50 μ m wide channels and was designed using AutoCAD software (San Rafael, CA) and printed as a high-resolution (20 000 dip) photomask (CAD/Art Services, OR). Negative-type photoresist (SU-8 2025, Microchem, Newton, MA) was spin-coated onto a 3 inch silicon wafer at 1200 rpm for 30 s to a thickness of 25 μ m. The mold was spin-coated with degassed PDMS pre-polymer solution at 800 rpm for 30 s and then kept at 70°C for 15 min. With the help of a "holding" magnet, two 2 mm diameter rare earth magnets were placed above the side channels where the magnetic microbeads were initially packed. Degassed PDMS pre-polymer solution was poured onto the assembly and baked for 2 h at 70°C. The assembly was peeled off from the mold. Holes (3 mm diameter) used as the sample and buffer reservoirs were punched. The PDMS assembly was irreversibly sealed to a glass slide after treating with oxygen plasma (Fig. 1A). The channel surface was coated following the method described by Wu *et al.* [22].

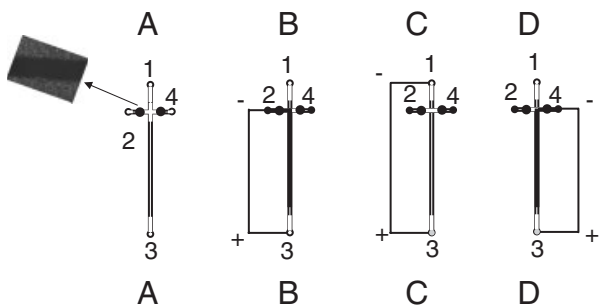


Figure 1. (A) Microchip and representative microchannel. (B–D) Microchip frontal analysis chromatography (MFAC) protocol (1, buffer well; 2, sample well; 3 waste well; 4, control well).

A solution of 20 mM HEPES (pH 7.40) containing 0.10% Tween-20 was used as the running buffer. The channel was rinsed with distilled water and running buffer, respectively, for 3 min. Aliquots (1.4 μ L) of the suspension of carboxylic acid-terminated magnetic beads and Teic-beads were introduced into the respective wells and were manipulated into the respective channels using a peristaltic pump. The channel was rinsed with running buffer in both directions at increasing speed to firmly pack the beads.

Figure 1B–D shows the voltage application sequence for the FAC process. A voltage of 1.0 kV was applied to the Teic column (Fig. 1B) for 300 s, followed by application of a voltage of 1.0 kV to the buffer and waste wells (Fig. 1C) for 100 s, and finally a voltage of 1.0 kV to the control column (Fig. 1D) for 300 s.

EOF was measured with running buffer using the current monitoring method described by Huang *et al.* [23]. Both the affinity and control columns were found to have the same EOF. Figure 2 shows the fluorescence images of the affinity and control columns after a solution of **1** (0.10 mg/mL) was washed through the microchannel for 6 min followed by a buffer rinse for 3 min. The control column showed no adsorption of **1**, whereas the affinity

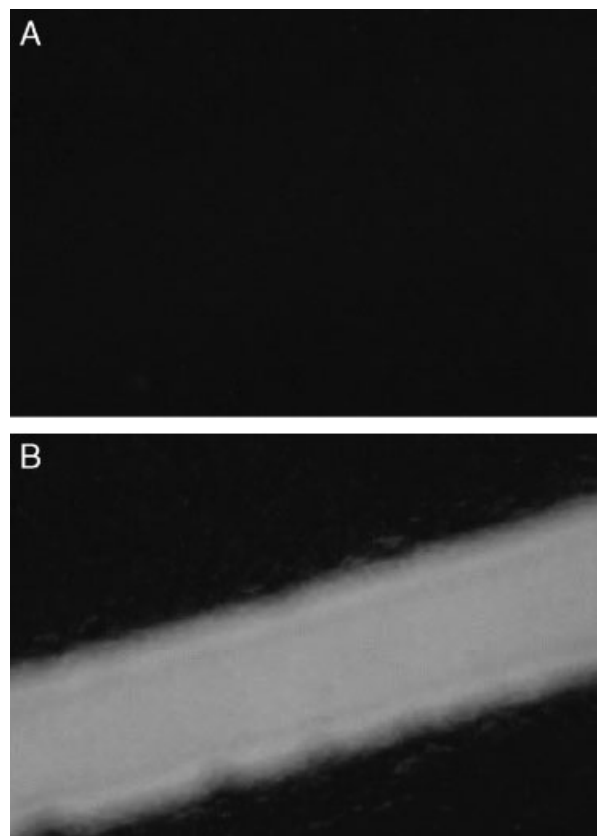


Figure 2. Fluorescence micrographs of (A) control channel and (B) affinity channel after incubation with **1** and after rinsing with the running buffer.

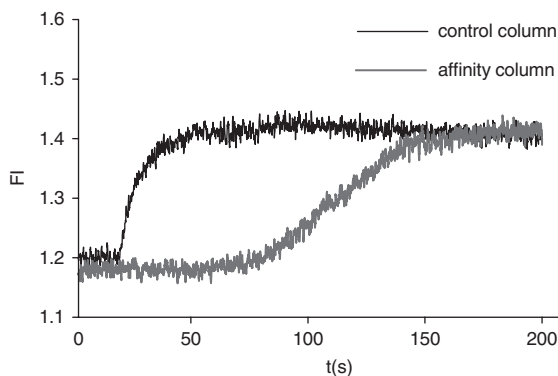


Figure 3. Representative electropherogram from a single binding measurement.

column did, proving that the binding of **1** to the Teic-beads was specific.

In this study, we first examined the binding of 80 μM **1** to the columns. Figure 3 is a representative electropherogram for a single run. The migration time and profile of **1** in the affinity column was different from those realized in the control column under the same conditions. The migration time and slope of the curve for **1** in the affinity column is greater and smaller, respectively, than that found in the control column. We attribute these differences to the fact that **1** binds to the Teic-beads and has no affinity for unmodified beads. The amount of bound peptide can be calculated from

$$q = \frac{L}{t_0} (t_1 - t_0) * A * C \quad (1)$$

Here, q , L , t_0 , t_1 , A , and C were the amount of bound **1**, effective separation length, the migration time in the control column, the migration time in the affinity column, the intersection area of the channel, and the sample concentration, respectively. For a single run, the sample consumption was estimated to be approximately 0.4 μL , whereas conventional FAC usually requires several mL for the binding assay.

To estimate the binding constant, six different sample concentrations were used (Fig. 4A). Scatchard analysis was used to linearize the binding data and is expressed as

$$r/C = -kr + nk \quad (2)$$

Here, r is the ratio of bound **1** to total Teic-beads, C is the concentration of **1**, k is the apparent binding constant, and n is the number of binding sites present on a Teic-bead. Substitution of Eq. (1) into Eq. (2) yields, after simplification

$$(t_1 - t_0)/t_0 = -kC(t_1 - t_0)/t_0 + nk/(ALq_T) \quad (3)$$

Here, q_T stands for the total amount of Teic-beads used in this study. Figure 4B is a plot of the binding of **1** to the Teic-beads according to Eq. (3). The binding constant was calculated to be $5.4 \times 10^4 \text{ M}^{-1}$. The value was in agreement with previous work [21].

We have described an electrokinetically miniaturized MFAC system for estimating the binding of a fluorescent

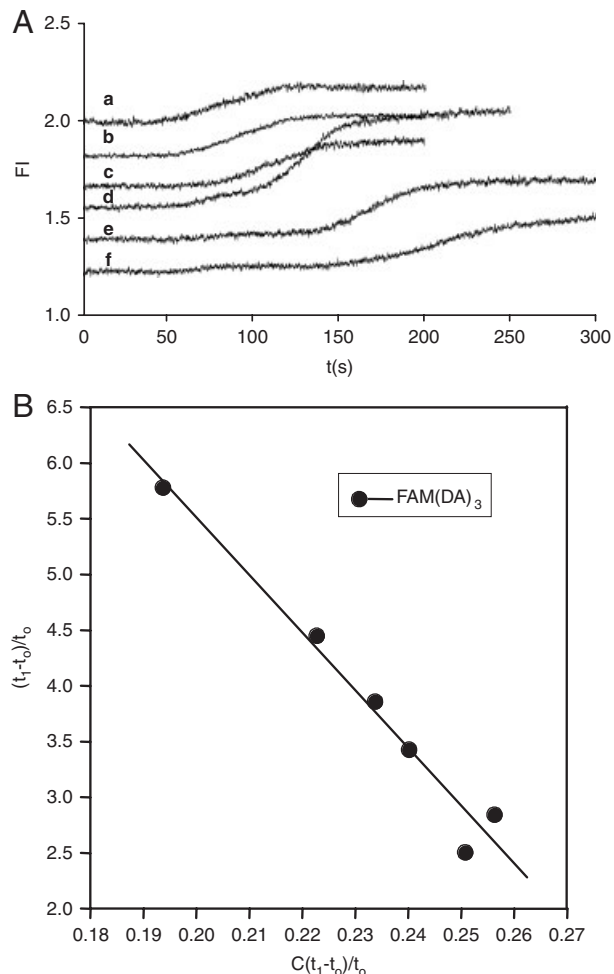


Figure 4. (A) Representative electropherograms used for the measurement of the binding constant between **1** and Teic-beads. (**1**) = a, 100 μM ; b, 90 μM ; c, 80 μM ; d, 70 μM ; e, 60 μM ; f, 50 μM). (B) Scatchard plot of the data for **1** and Teic-beads.

ligand to the glycopeptide antibiotic Teic. Compared with conventional FAC, this technique offers several advantages including extremely low sample consumption, ease of packing, and reproducibility. Further work involves the development of other microfluidic-based FAC techniques and the analysis of other receptor–ligand interactions.

The authors gratefully acknowledge financial support for this research by grants from the National Science Foundation (CHE-0515363, DMR-0351848 and CBET-0723271).

The authors have declared no conflict of interest.

References

- [1] Cuatrecasas, P. W. M., Anfinsen, C. B., *Proc. Natl. Acad. Sci. USA* 1968, **61**, 636–643.

- [2] Hofstetter, H., Hofstetter, O., Schurig, V., *J. Microcolumn Sep.* 1998, 10, 287–291.
- [3] Hage, D. S., *J. Chromatogr. B* 1998, 715, 3–28.
- [4] Slys, G. W., Schriemer, D. C., *Rapid Commun. Mass Spectrom.* 2003, 17, 1044–1050.
- [5] Zhang, B., Palcic, M. M., Schriemer, D. C., Alvarez-Manilla, G., Pierce, M., Hindsgaul, O., *Anal. Biochem.* 2001, 299, 173–182.
- [6] Chan, N. W. C., Lewis, D. F., Hewko, S., Hindsgaul, O., Schriemer, D. C., *Comb. Chem. High Throughput Screen.* 2002, 5, 395–406.
- [7] Chan, N. W. C., Lewis, D. F., Rosner, P. J., Kelly, M. A., Schriemer, D. C., *Anal. Biochem.* 2003, 319, 1–12.
- [8] Ohshima, Y., Kasai, K.-i., Nomoto, H., Inoue, Y., *J. Biol. Chem.* 1985, 11, 6882–6887.
- [9] Besanger, T. R., Hodgson, R. J., Guillon, D., Brennan, J. D., *Anal. Chim. Acta* 2006, 561, 107–118.
- [10] Duffy, D. C., McDonald, J. C., Schueller, O. J. A., Whitesides, G. M., *Anal. Chem.* 1998, 70, 4974–4984.
- [11] Giordano, B. C., Jin, L. J., Couch, A. J., Ferrance, J. P., Landers, J. P., *Anal. Chem.* 2004, 76, 4705–4714.
- [12] Ludwig, M., Kohler, F., Belder, D., *Electrophoresis* 2003, 24, 3233–3238.
- [13] Tian, H. J., Emrich, C. A., Scherer, J. R., Mathies, R. A., Andersen, P.S., Larsen, L.A., Christiansen, M., *Electrophoresis* 2005, 26, 1834–1842.
- [14] Schwarz, M. A., Hauser, P. C., *J. Chromatogr. A* 2001, 928, 225–232.
- [15] Chiem, N. H., Harrison, D. J., *Electrophoresis* 1998, 19, 3040–3044.
- [16] Vlckova, M., Stettler, A. R., Schwarz, M. A., *J. Liq. Chromatogr. Relat. Technol.* 2006, 29, 1047–1076.
- [17] Stettler, A. R., Schwarz, M. A., *J. Chromatogr. A* 2005, 1063, 217–225.
- [18] Liu, X., Liu, X., Liang, A., Shen, Z., Zhang, Y., Dai, Z., Xiong, B., Lin, B., *Electrophoresis* 2006, 27, 3125–3128.
- [19] Le Saux, T., Hisamoto, H., Terabe, S., *J. Chromatogr. A* 2006, 1104, 352–358.
- [20] Liu, X., Liang, A., Shen, Z., Liu, X., Zhang, Y., Dai, Z., Xiong, B., Lin, B., *Electrophoresis* 2006, 27, 5128–5131.
- [21] Liu, X., Gomez, F. A., *Anal. Bioanal. Chem.* 2009, 393, 615–621.
- [22] Wu, D. P., Qin, J. H., Lin, B. C., *Lab Chip* 2007, 7, 1490–1496.
- [23] Huang, X., Gordon, M. J., Zare, R. N., *Anal. Chem.* 1998, 60, 1837–1838.