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A capillary electrophoresis mobility shift assay for protein–DNA binding affinities free in solution

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ABSTRACT

Quantitative determination of dissociation constants for DNA–protein complexes will help clarify the molecular mechanisms of transcription, replication and DNA repair. A practical capillary electrophoresis mobility shift assay (CEMSA) for protein–DNA affinities free in solution is presented. The method is fast and simple, precise and general. The speed (<2 min separations) and simplicity derive from the use of an uncoated capillary with no gel matrix. The dissociation constant for GCNK58, a DNA-binding-region construct of the yeast transcription factor GCN4, binding to the AP1 DNA site was measured (K_d = 35 ± 4 nM) to demonstrate the utility of the method.

The quantitative assessment of DNA–protein affinities is essential to understanding transcription, the beginning of biological processes including normal cellular function, development and many diseases (1). Existing art for the determination of DNA-binding affinities is the electrophoresis mobility shift assay (EMSA) (2). A capillary electrophoresis mobility shift assay (CEMSA) (3) for the precise determination of protein–DNA dissociation constants free in solution is described in this paper. The key features of the method are the rapid separation and quantitation of complex and free DNA, in uncoated capillaries with no gel matrixes, using high sensitivity laser-induced-fluorescence (LIF) detection of fluorescein-labeled DNA. The caging effect of the gel matrix on dissociation constant measurements (4) is absent in this method. Similar K_d values are observed as in EMSA (5) and microcalorimetry (6).

Capillary electrophoresis (CE) separates analytes on the basis of their mass-to-charge ratio. Our method takes particular advantage of the electroosmotic flow generated by the negatively charged silanol groups on the capillary walls at ≥pH 6 (7). This CE assay uses the reverse polarity of EMSA, and the order of elution is reversed: free protein, protein/DNA complex, then DNA.

CEMSA has the advantages of automation, high resolution, fast assay times, on-line detection and the use of small amounts of precious samples. CE has been applied to the study of DNA–protein complexes using fluorescently-labeled DNA with LIF detection, but not free in solution (8–10). In our method, 2 min separations in short, uncoated capillaries, without sieving matrixes, are coupled with precise quantitation of K_d values. We used the yeast transcriptional activator GCNK58 construct (11) as the prototype for measuring DNA-binding affinity by the new CEMSA method. The method is general for different classes of DNA-binding proteins, including zinc-finger and basic-helix–loop–helix-leucine-zipper (b-HLH-ZIP; G.J.Foulds and F.A.Etzkorn, unpublished results).

Excellent separation of the free AP1, complex and GCNK58 was obtained using CE in uncoated, gel-free capillaries with LIF detection of fluorescein-labeled DNA. In a benchmark assay, we measured the dissociation constant of the DNA-binding protein GCNK58 and a 20 bp oligonucleotide including the recognition site AP1 (11).

For CEMSA, the zwitterionic buffer 3-(*N*-morpholino)–propane sulfonic acid (MOPS) with the soft counterion triethylamine (Et_3N) was found to yield better peak shape and resolution with low current at high voltage (25 kV) than the phosphate buffer used in the EMSA of GCNK58 (5). High voltage gave fast separation and low current gave less Joule heating, allowing the complex to stay intact during electrophoresis. The difference in ionic strength between the sample buffer (50 mM MOPS·Et_3N, 50 mM KCl) and the run buffer (10 mM MOPS·Et_3N) helped maintain the original concentration of complex during the separation (2). The minimum-length capillary column that fits the Beckman CE cartridge (27 or 20 cm from inlet to detector) was used. The ratio of complex peak area to total DNA (R, see below) was found to be independent of the applied voltage at 25 kV and below, so 25 kV was used as the standard applied voltage in our CEMSA. Adsorbed ions were removed and the charged capillary wall was regenerated by washing with 0.1 M NaOH and run buffer between runs.

Figure 1 shows the set of electropherograms obtained at varying concentrations of GCNK58 and 1 nM AP1. The saturation of AP1 (R), defined as:

$$R = [GCNK58_2{\cdot}AP1]/\{[GCNK58_2{\cdot}AP1] + [AP1]\},$$

was plotted against increasing concentrations of GCNK58 (Fig. 2). For determination of K_d, the data were fit to equation **1**:

$$R = 0.5[GCNK58]/\{K_d + 0.5[GCNK58]\} \quad \mathbf{1}$$

The equilibrium dissociation constant for homodimer–DNA complex formation, K_d, was found to be 35 ± 4 nM. This value is the same as determined by isothermal calorimetry in the sample buffer: 50 mM Tris–HCl, pH 7.5, 10 mM NaCl, 10 mM $MgCl_2$ (35.1 ± 8.9 nM) (6), and reasonably close to a K_d value previously determined by EMSA in 50 mM K_xPO_4 pH 7.5, 50 mM KCl (2×10^{-8} M) (5), both without non-specific DNA included in the

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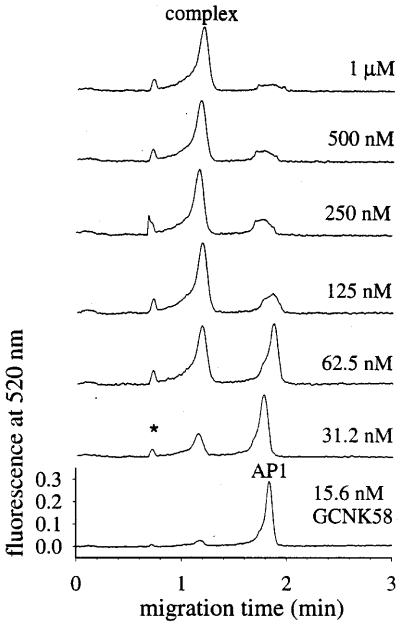

Figure 1. Electropherograms of AP1 at 1 nM and GCNK58 at the indicated concentrations in 50 mM MOPS·Et_3N pH 7.5, 50 mM KCl, 133 μg/ml poly(dA-dT)·poly(dA-dT), 100 μg/ml bovine serum albumin, 5% glycerol. DNA was synthesized (Biomolecular Research Facility, University of Virginia) with 5′-carboxyfluorescein phosphoramidite (Glen Research) attached. d(AAACTGGATGAGTCATAGGA) (3 nM) and Xd(TTCCTATGACTCATC-CAGTT) (2 nM) where X is fluorescein (AP1), were freshly annealed at 90°C for 5 min and cooled slowly to room temperature. Serial dilutions of GCNK58 were mixed with an equal volume (10 μl) of annealed AP1 and preincubated at 4°C for 2–3 h. CE was performed on a Beckman P/ACE 2100 with Laser Module 488, LIF detector and System Gold software (v. 8.1) in 75 μm ID uncoated capillaries (Polymicro Technologies), 27 cm long, 20 cm effective length to the detector, with a cooled sample tray. Before runs, the capillary was rinsed for 2 min with 0.1 M NaOH and 2 min with run buffer: 10 mM MOPS·Et_3N pH 7.5. Samples were injected for 2 s at low pressure and electrophoresed at 25 kV in run buffer at 22°C, at normal CE polarity (cathode at the detector end).

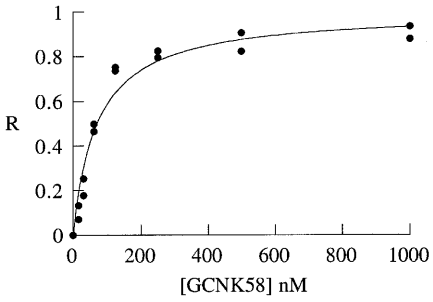

Figure 2. Saturation of AP1 (R) versus concentration of GCNK58. K_d was calculated to be 35 ± 4 nM by non-linear least squares fit of the data to equation **1** using KaleidaGraph v. 3.0.5.

sample buffer. Inclusion of the non-specific DNA, poly(dA·dT), gives a K_d value representative of specific DNA binding only (12).

In the absence of the non-specific DNA, as in the EMSA (5), a lower K_d value of 1.6 ± 0.2 nM was obtained (data not shown). In this case, cooperativity of dimerization and DNA-binding was observed. We used equation **2**:

$$R = (570\ nM)^{-1}[GCNK58]^2/\{K_d + (570\ nM)^{-1}[GCNK58]^2\} \quad \mathbf{2}$$

including the dimerization constant of 0.57 ± 0.19 μM (13) to fit the data. This result is unexpected in light of observations that sequestration (14) and caging (4) effects stabilize some protein/DNA complexes during gel electrophoresis (15). The fast separations of our assay may prevent dissociation of the complex even in the absence of gel matrix.

We have developed a fast, precise and practical CEMSA for DNA–protein affinities. Each concentration was analyzed in 3 min with a 4 min wash cycle. A series of six concentrations was performed in duplicate in ~85 min on a single column CE instrument. The assay uses open, uncoated capillaries, no radioactivity and an automatic sample tray, permitting hands-off assays to be performed routinely. The measured affinity of the GCNK58/AP1 complex was 35 ± 4 nM.

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REFERENCES

1 Maddox,J. (1994) *Nature*, **368**, 95.
2 Garner,M.M. and Revzin,A. (1981) *Nucleic Acids Res.*, **9**, 3047–3060.
3 Heegaard,N.H.H. and Robey,F.A. (1993) *J. Liq. Chromatogr.*, **16**, 1923–1939.
4 Fried,M.G. and Crothers,D.M. (1981) *Nucleic Acids Res.*, **9**, 6505–6525.
5 Weiss,M.A., Ellenberger,T., Wobbe,C.R., Lee,J.P., Harrison,S.C. and Struhl,K. (1990) *Nature*, **347**, 575–578.
6 Berger,C., Jelesarov,I. and Bosshard,H.R. (1996) *Biochemistry*, **35**, 14984–14991.
7 Grossman,P.D. and Colburn,J.C. (1992) *Capillary Electrophoresis: Theory and Practice*. Academic Press, San Diego.
8 Xian,J., Harrington,M.G. and Davidson,E.H. (1996) *Proc. Natl Acad. Sci. USA*, **93**, 86–90.
9 Stebbins,M.A., Hoyt,A.M., Sepaniak,M.J. and Hurlburt,B.K. (1996) *J. Chromatogr. B*, **683**, 77–84.
10 Janini,G.M., Fisher,R.J., Henderson,L.E. and Issaq,H.J. (1995) *J. Liq. Chromatogr.*, **18**, 3617–3628.
11 Ellenberger,T.E., Brandl,C.J., Struhl,K. and Harrison,S.C. (1992) *Cell*, **71**, 1223–1237.
12 Ausubel,F.M., Brent,R., Kingston,R.E., Moore,D.D., Seidman,J.G., Smith,J.A. and Struhl,K. (eds) (1992) *Short Protocols in Molecular Biology*. 2nd Ed. Greene Publishing Assoc. and John Wiley & Sons, NY, pp. 12–5 to 12–7.
13 Wendt,H., Baici,A. and Bosshard,H.R. (1994) *J. Am. Chem. Soc.*, **116**, 6973–6974.
14 Fried,M.G. and Liu,G. (1994) *Nucleic Acids Res.*, **22**, 5054–5059.
15 Lane,D., Prentki,P. and Chandler,M. (1992) *Microbiol. Rev.*, **56**, 509–528.