Affinity Capillary Electrophoresis

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Introduction and Principle

Affinity capillary electrophoresis (ACE) is a new procedure for studying protein–ligand interactions.[1-9] Its potential applications include developing tight-binding drug candidates, screening libraries for lead compounds, characterizing the effective charges of proteins, and measuring enzymatic activities. We will illustrate some of these applications using several examples from our work, with a focus on a model system comprising carbonic anhydrase and arylsulfonamides. Most of our work has been carried out using uncoated fused silica capillaries. In principle, proteins can be examined by capillary electrophoresis using conditions that maintain them in their native, functional forms. In practice, adsorption of proteins (especially those with high molecular weight and p*I*) to the wall of the uncoated capillaries is an important limitation to the generality of the method. Less adsorptive, coated capillaries are now becoming available, but are not yet in wide use.

Capillary Electrophoresis. Capillary electrophoresis (CE) differentiates charged species on the basis of mobility under the influence of an electric field gradient.[10,11] The value of the electrophoretic mobility μ of a species is directly related to its net charge and inversely related to its hydrodynamic drag. Eq 1 is

$$\mu \cong C_P \frac{Z}{M^\alpha} \tag{1}$$

$$\mu = \frac{L_t L_d}{V}\left(\frac{1}{t_P} - \frac{1}{t_{EO}}\right) \tag{2}$$

an approximate expression of this relationship: here, C_P is a constant for a given protein (P) of mass M and charge Z. Experimentally, the electrophoretic mobility is determined by the migration time of a protein (t_P) and a neutral marker (t_{EO}), where L_d is the length of the capillary from injection end to the detector, L_t is the total length of the capillary, and V is the voltage that is applied across the capillary (eq 2).

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Pioneering works by Karger,[12] Grossman,[13] Regnier,[14] Novotny,[15] Jorgenson,[16] and others influenced the rapid opening of the field of CE to commercialization; the development of versatile and relatively inexpensive commercial instruments now makes it routinely accessible as a technique in analytical biochemistry.

Affinity Capillary Electrophoresis. ACE measures the value of electrophoretic mobility $\mu_{P,L}$ for a protein (P) as a function of concentration of a ligand (L) that binds to it and is present in the electrophoresis buffer (Figure 1).[1-9] ACE is related to gel retardation assays used in protein biochemistry,[17] but takes advantage of the high resolving power of CE.

One can measure the binding constant (K_b) of a ligand to the protein using Scatchard analysis (eq 3) where $\Delta\mu_{P,L}$ is the change in mobility of the protein P at a given concentration of the ligand L ($\mu_{P,L}$) compared to that without the ligand (μ_P) in the electrophoresis buffer, and $\Delta\mu_{P,L}^{max}$ is the change in mobility at a

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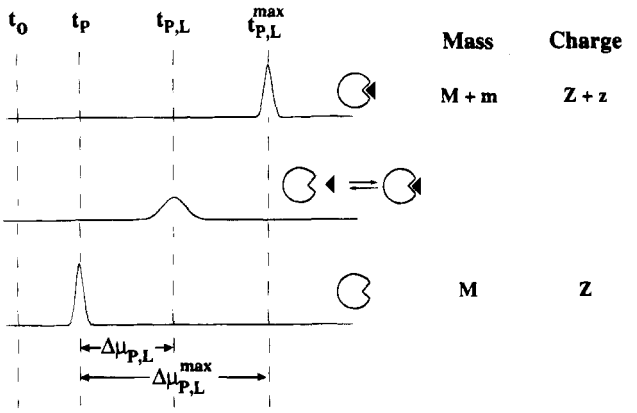

Figure 1. The electrophoretic mobility of a protein (mass M and charge Z) varies with the extent to which the binding site is occupied by a ligand (solid triangle) of mass m and charge z during electrophoresis. Peak broadening is observed when $(k_{off})^{-1}$ is comparable to the emergence time.

$$\Delta\mu_{P,L}/[L] = K_b\Delta\mu_{P,L}^{max} - K_b\Delta\mu_{P,L} \quad (3)$$

$$\Delta t_L/[L] = K_b\Delta t^{max} - K_b\Delta t_L \quad (4)$$

concentration of ligand L that will saturate the active site of the protein. Measuring changes in migration time of the protein (Δt_L and Δt_L^{max}) as approximations of changes in electrophoretic mobilities ($\Delta\mu_{P,L}$ and $\Delta\mu_{P,L}^{max}$) can provide an estimation of binding constants (eq 4) in cases where the electroosmotic flow (EOF) does not change significantly on the addition of the ligand(s) to the electrophoresis buffer (see section on correction for EOF). Assumptions associated with eqs 3 and 4 include the following: (a) the binding interaction is monovalent; (b) the quantity of receptor is much lower than the total quantity of ligand available for binding; (c) equilibrium is achieved between bound and unbound species; (d) the interaction of the ligand and the receptor with the capillary wall does not significantly alter the binding of the ligand to the receptor; and (e) the electric field does not influence the binding constants.

In principle, there are two limiting ways by which a ligand could change the electrophoretic mobility of the protein upon binding (Figure 1): by changing the charge, while leaving the hydrodynamic drag unchanged (i.e., small, charged ligands), or by changing the hydrodynamic drag, while leaving the charge unchanged (i.e., large, neutral ligands). For a number of technical reasons, we have focused on the former strategy.

Model System for ACE: Binding of Arylsulfonamides to Carbonic Anhydrase

We chose carbonic anhydrase[18] (CA; EC 4.2.1.1, from human and bovine erythrocytes) as a model protein because (1) it does not adsorb significantly to the wall of uncoated capillaries, (2) it is available commercially,

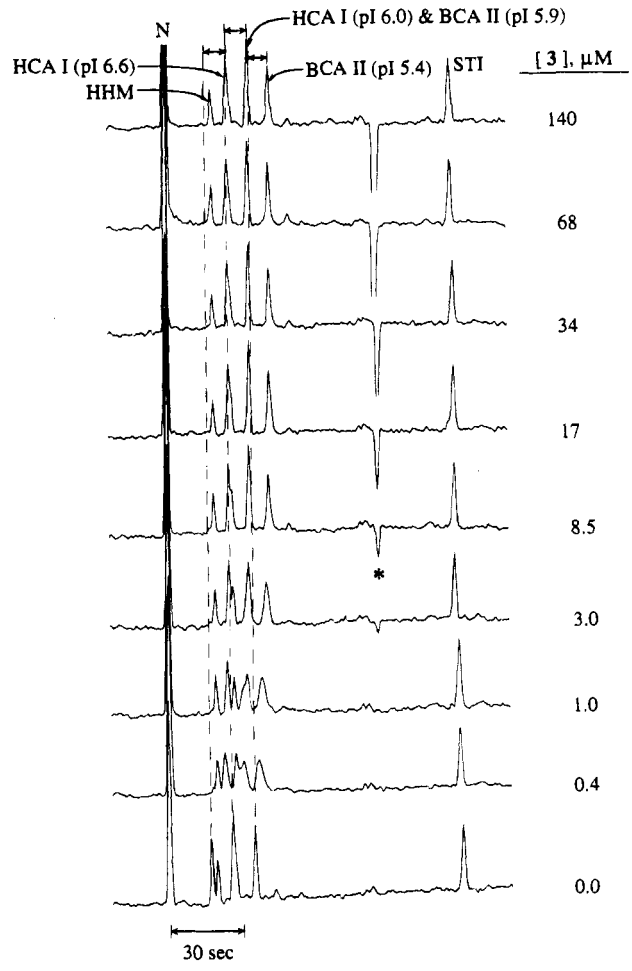

Figure 2. Affinity capillary electrophoresis of a mixture of isozymes of carbonic anhydrase: HCA I (human carbonic anhydrase, pI 6.6), HCA I (pI 6.0). BCA II (bovine carbonic anhydrase, pI 5.9), and BCA II (pI 5.4). The electrophoresis buffer used was tris (25 mM)-glycine (192 nM) at pH 8.3. MO is added to measure EOF; HHM and STI are protein markers that do not interact with affinity ligand **3**. The inverted peak (*) migrates at the emergence time of free **3** and is due to the lack of **3** in the injecting samples.

(3) its crystal structure is known,[19] (4) it exists as a number of isozymes that differ in pI, (5) many arylsulfonamides bind to it with values of K_b between 10^5 and 10^9 M^{-1}, and (6) charged derivatives of these ligands are easy to synthesize.[1,2]

Figure 2 shows electropherograms of a mixture of CA isozymes (from human and bovine erythrocytes) in an electrophoresis buffer containing a monocarboxylated arylsulfonamide ligand **3**. Mesityl oxide (MO) was added in the sample to correct for EOF; horse heart myoglobin (HHM) and soybean trypsin inhibitor (STI) were used as noninteracting protein standards. The migration times of MO, HHM, and STI are independent of the concentration of **3** (up to 140 μM). Only the isozymes of CA change their electrophoretic mobilities upon binding **3**. Scatchard analysis of the changes in electrophoretic mobilities gives the binding affinity of the ligand to different isozymes simultaneously (next section).

Quantitative Measurement of Binding Constants. Figure 3A shows a set of electropherograms

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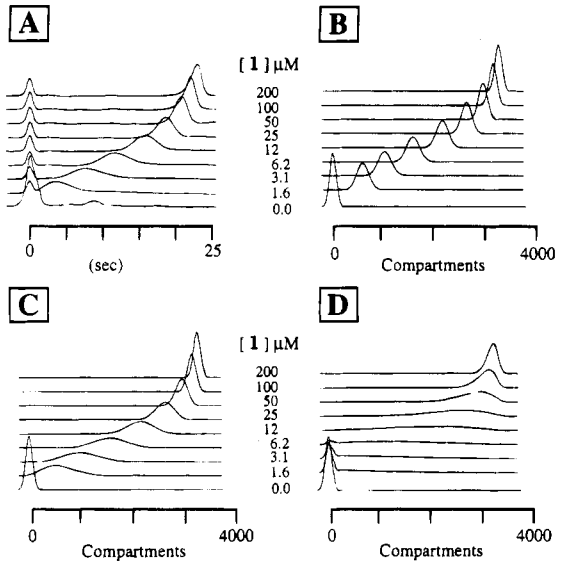
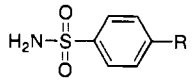
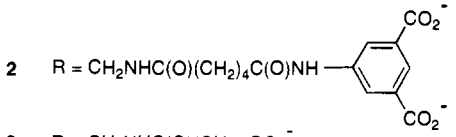


Figure 3. The migration time of BCA II (p*I* 5.9) changes with increasing concentration of the tricarboxylated arylsulfonamide ligand **1** in the electrophoresis buffer. Stacked electropherograms A were obtained experimentally ([BCA II] = 10 μM in tris-glycine buffer, pH 8.3). The nonmobile peak is due to HHM, used as the noninteracting protein standard. Panels B–D were generated by simulation. Simulation parameters k_{off} (s^{-1}), k_{on} (10^5 M^{-1} s^{-1}): (B) 1.0, 1.5; (C) 0.10, 0.15; (D) 0.010, 0.015. In the simulation, the concentration in each compartment was adjusted every 10 ms and the contents of the compartments for the mobile complex were transferred every 100 ms.



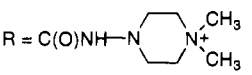
1 R = $CH_2NHC(O)(CH_2)_4C(O)NHC[CH_2O(CH_2)_3SCH_2CO_2^-]_3$



2 R = $CH_2NHC(O)(CH_2)_4C(O)NH$–(3,5-CO_2^-)

3 R = $CH_2NHC(O)(CH_2)_4CO_2^-$

4 R = H



5 R = $C(O)NH$–(piperazinium, CH_3, CH_3)

for bovine CA II (BCA II, p*I* 5.9) at increasing concentrations of arylsulfonamide ligand **1** having three carboxylic acid groups present in the electrophoresis buffer. The peak shift is accompanied by significant peak broadening due to a slow dissociation of the complex (see below in the section on kinetics). Analysis of these data using a Scatchard plot (eq 3) gave a value of $K_b = 1.5 \times 10^5$ M^{-1} that agreed well with a value, $K_b = 1.4 \times 10^5$ M^{-1}, obtained by a fluorescence-based assay.[2] The noninteracting neutral marker and noninteracting protein standards are included in the sample to correct for changes in EOF with changes in concentration of the ligand. They also detect changes in mobility due to nonspecific interaction between proteins and ions.

We have also successfully applied ACE to other binding systems including calmodulin (from bovine testes) and calcium ion, glucose-6-phosphate dehydrogenase (G6PDH) and $NADP^+$, SH3 domain and peptides, monoclonal antibody (anti DNP) and *N*-dinitrophenylaminocaproic acid, and thrombin and peptides.

Correction for Changes in Electroosmotic Flow. The binding constants of charged ligands to proteins can be measured by ACE even under conditions in which the migration times are affected by changes in EOF resulting from changes in concentrations of ligands. These corrections are made by observing the behavior of an electrically neutral internal standard and checked by observing noninteracting proteins included in the sample.[6] In eqs 5–7, the experimental

$$\mu = \mu_{P,L} - \mu_P \tag{5}$$

$$= \frac{L_t L_d}{V}\left[\left(\frac{1}{t_{P,L}} - \frac{1}{t_{EO,L}}\right) - \left(\frac{1}{t_P} - \frac{1}{t_{EO}}\right)\right] \tag{6}$$

$$\approx \frac{L_t L_d}{V}\frac{\Delta t}{t^2} \tag{7}$$

parameters $t_{P,L}$, t_P, $t_{EO,L}$, and t_{EO} are the measured migration times of protein (P) and neutral marker (EO) peaks in the presence and the absence of the ligand (L) in the electrophoresis buffer, respectively. Binding constants based on corrected electrophoretic mobility for the protein can then be determined using eq 6 in combination with eq 3. When the change in EOF of the system is negligible over the concentration range used in the analysis, Δt ($t_{P,L} - t_P$) is proportional to $\Delta\mu_{P,L}$ (eq 6), and eq 4 can be used to estimate the binding constant.

Representative examples of systems requiring corrections to changes in EOF are the interactions of CA with positively charged arylsulfonamides[6] and SH3 domain–proline–rich peptides.[7] Representative examples of those not requiring EOF corrections are the interactions of CA with negatively charged arylsulfonamides.[12]

Kinetics of Association and Dissociation ($K_b = k_{on}/k_{off}$) and Electrophoretic Peak Shapes. Besides changes in the electrophoretic mobility upon binding, the peak for the protein sometimes also broadens in the region of intermediate mobility (Figure 3A).[2,20] This type of broadening reflects equilibration between free protein and protein–ligand complex having different electrophoretic mobilities. The peak width depends on the value of k_{off} of the protein–ligand complex and the run time of each electrophoresis experiment. In the system of CA and sulfonamide ligands, 10–100 association and dissociation events occur during each electrophoresis run.[2,20]

We were able to extract the rate constants from the peak widths by simulating the behavior of the protein under conditions of the ACE experiment (Figure 3B–D). Our simulation of ACE suggests that these experimental electropherograms can be explained in terms of relatively few variables: on and off rates (and thus, binding constant), concentration of the ligand(s), and relative mobilities of the protein and its complex(es). The simulation assumed the interaction of the protein with the capillary wall to be negligible. This potential of ACE to determine the kinetic and

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equilibrium constants simultaneously for protein–ligand interactions is especially attractive in evaluating drug candidates.[21,22]

Binding of Carbonic Anhydrase with Two Ligands, One Electrically Neutral and the Other Charged. Direct analysis of the binding interaction of CA with an electrically neutral, low molecular weight arylsulfonamide **4** is not possible using the format of ACE we have described, because the electrophoretic mobilities of free and bound proteins are experimentally indistinguishable. We have previously shown that one way of circumventing this limitation is to measure the binding constant of the neutral ligand **4** as it competes with charged ligand **1** of known binding constant.[2]

Other Binding Interactions

Protein Charge Ladders. ACE can also be used to estimate the effective charges of proteins in solution at a given pH.[9] These applications permit convenient estimation of effective charge for a protein in solution without knowledge of its composition or amino acid sequence, and they require only very small amounts of protein. We have examined two methods for generating a charge ladder, that is, a series of derivatives of a protein differing by known increments of charge but differing only minimally in hydrodynamic drag. One method is by noncovalent association of a protein with differently charged ligands; the second method modifies the proteins covalently (Figure 4). When the charge of a protein is modified from Z_0 to a value Z_n, the ratio of the mobilities of the modified and unmodified proteins is given by eq 8. Algebraic manipulation of eq 8 gives eq 9; this equation relates the emergence times of peaks in the charge ladder to the effective charge of the protein (here t_0 and t_n are the emergence times of the unmodified and modified proteins, respectively, and $\Delta t_n = t_n - t_{EO}$).

$$\frac{\mu_n}{\mu_0} = \frac{Z_n}{Z_0} \tag{8}$$

$$\Delta Z_n = Z_n - Z_0 = Z_0\left(\frac{\Delta t_n/t_n}{\Delta t_0/t_0} - 1\right) \tag{9}$$

Noncovalent modification of the charge of CA to generate a protein charge ladder is illustrated by obtaining the emergence times of complexes of BCA II with the arylsulfonamides **2**, **3**, and **5**. High concentrations (~millimoles/liter) of the ligands were used to ensure the saturation of the active site of BCA II. Analysis of the data using eq 8 indicates that $Z_0 \approx -3.7$ for BCA II (p*I* 5.9), and $Z_0 \approx -5.0$ for BCA II (p*I* 5.4). Covalent modification of BCA II (p*I* 5.9) and BCA II (5.4) is illustrated using reaction with 4-sulfophenyl isothiocyanate; the results are consistent with those from ACE studies: analysis of the charge ladders yields estimates of the effective charges at pH 8.3 that $Z_0 \approx -3.5$ for BCA II (p*I* 5.9), and $Z_0 \approx -4.5$ for BCA II (p*I* 5.4). The difference of one unit of charge between BCA II (p*I* 5.9, Arg-56) and BCA II (p*I* 5.4, Gln-56) observed experimentally is that expected from the amino acid sequences. Other proteins whose effective charge has been checked using this procedure include trypsin, insulin, myoglobin, α-lactalbumin,[9] and an anti-2,4-DNP antibody.[23]

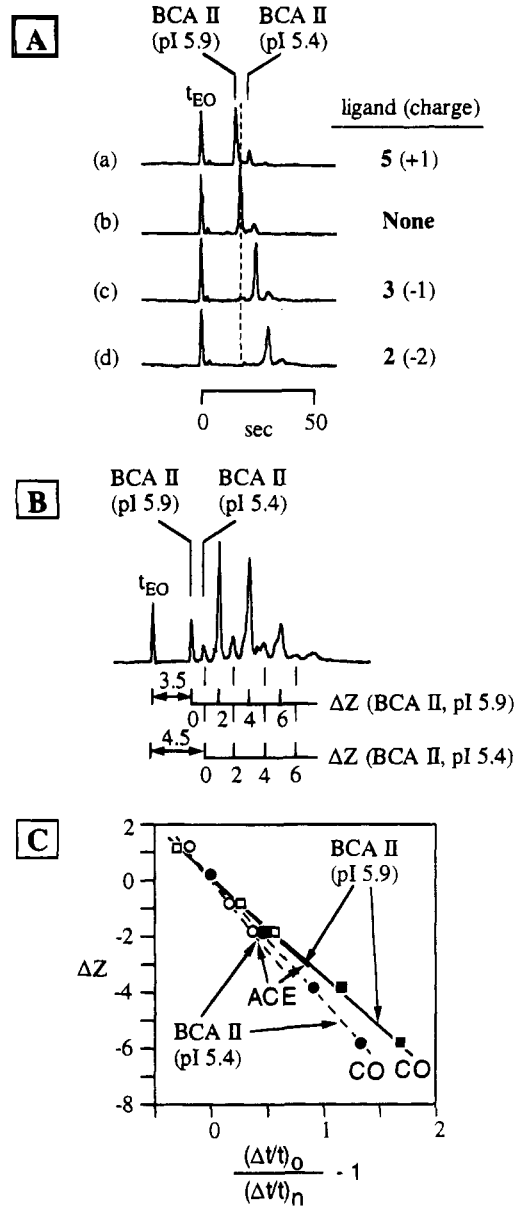

Figure 4. Measurement of effective charge of bovine carbonic anhydrases (BCA II). The sample contained two isozymes BCA II (p*I* 5.9) and BCA II (p*I* 5.4) in about a 10:1 ratio; these isozymes differ by one unit of charge. (A) The charge ladder was generated by complexation of BCA II with charged ligands: (a) [**5**] = 1.0 mM, (b) buffer, (c) [**3**] = 1.0 mM, and (d) [**2**] = 0.5 mM. (B) Covalent modification of lysine ϵ-amino groups on BCA II by 4-sulfophenyl isothiocyanate generated two independent charge ladders of BCA II (p*I* 5.9) and BCA II (p*I* 5.4) (buffer = tris (25 mM)-glycine (192 mM), pH = 8.3, $t_{EO} \approx$ 170 s). The emergence times of the unmodified proteins are indicated and provide origins of scales in Δ*Z*. The time scale applies to both A and B conditions of electrophoresis. (C) Determination of effective charge from the analysis of charge ladders using eq 9. The data shown for BCA II (p*I* 5.9) and BCA II (p*I* 5.4) were estimated using ladders generated by ACE and covalent (CO) methods.

Stoichiometry of Binding. ACE can also conveniently determine binding stoichiometries of receptor–

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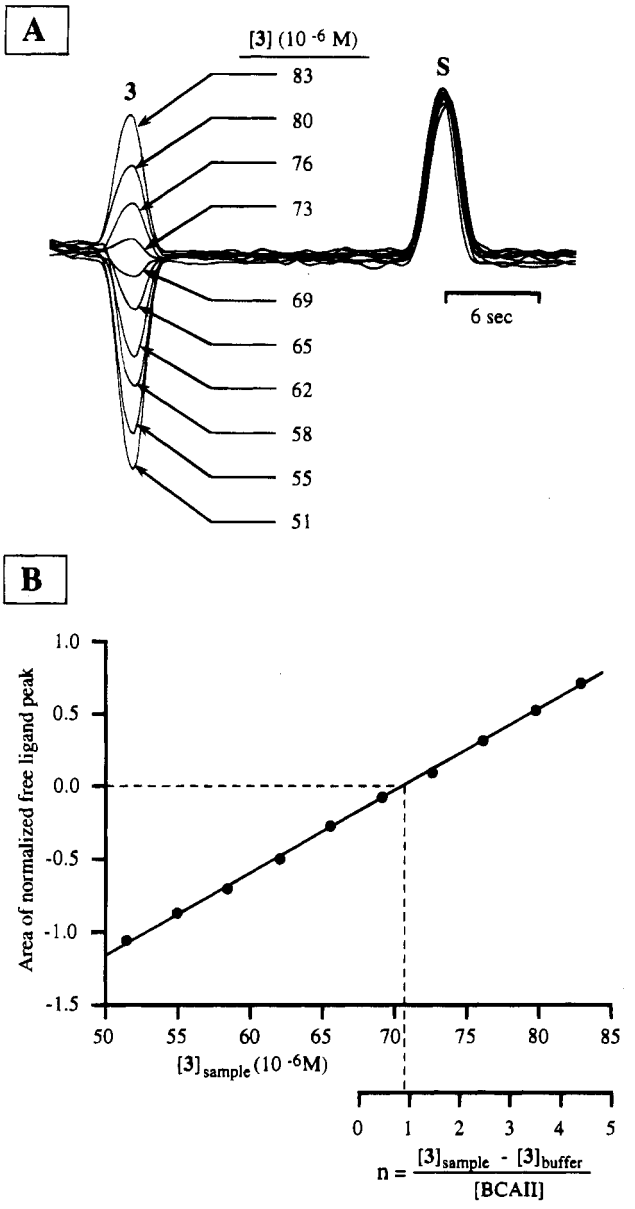


Figure 5. Determination of the stoichiometry of binding of a low-affinity system: carbonic anhydrase and arylsulfonamide **3**. (A) ACE of samples having a constant concentration of BCA II (p*I* 5.9; 4.2 μM) and **3** (67 μM) in tris-glycine buffer containing various concentrations of **3** (pH 8.3). The total electrophoresis time in each experiment was 2.5 min at 12 kV using 200 nm as the detection wavelength. 2-Iodobenzoic acid (S) was used as the internal standard for peak areas. (B) The graph summarizes experimental data derived from panel A. (Data from Walsh, C. T., et al.; ref 8.)

ligand interactions (Figures 5 and 6).[8] Figure 5 shows that BCA II binds the ligand **3** with a stoichiometry of 1:1. The interaction of CA with arylsulfonamides is an example of a low-affinity system.

The interaction of a mouse monoclonal IgG antibody (anti-HSA) to human serum albumin (HSA) is a representative example of a high-affinity system (Figure 6). For each mole of an anti-HSA molecule in the sample, addition of a stoichiometric amount (i.e., 2 mol) of the HSA results in the formation of 1 mol of the anti-HSA·HSA complex (Figure 6). ACE can also readily separate stable intermediate species corresponding to 1:1, 1:2, and 1:3 complexes obtained when streptavidin (from *Streptomyces avidinii*) binds a derivative of biotin.[8]

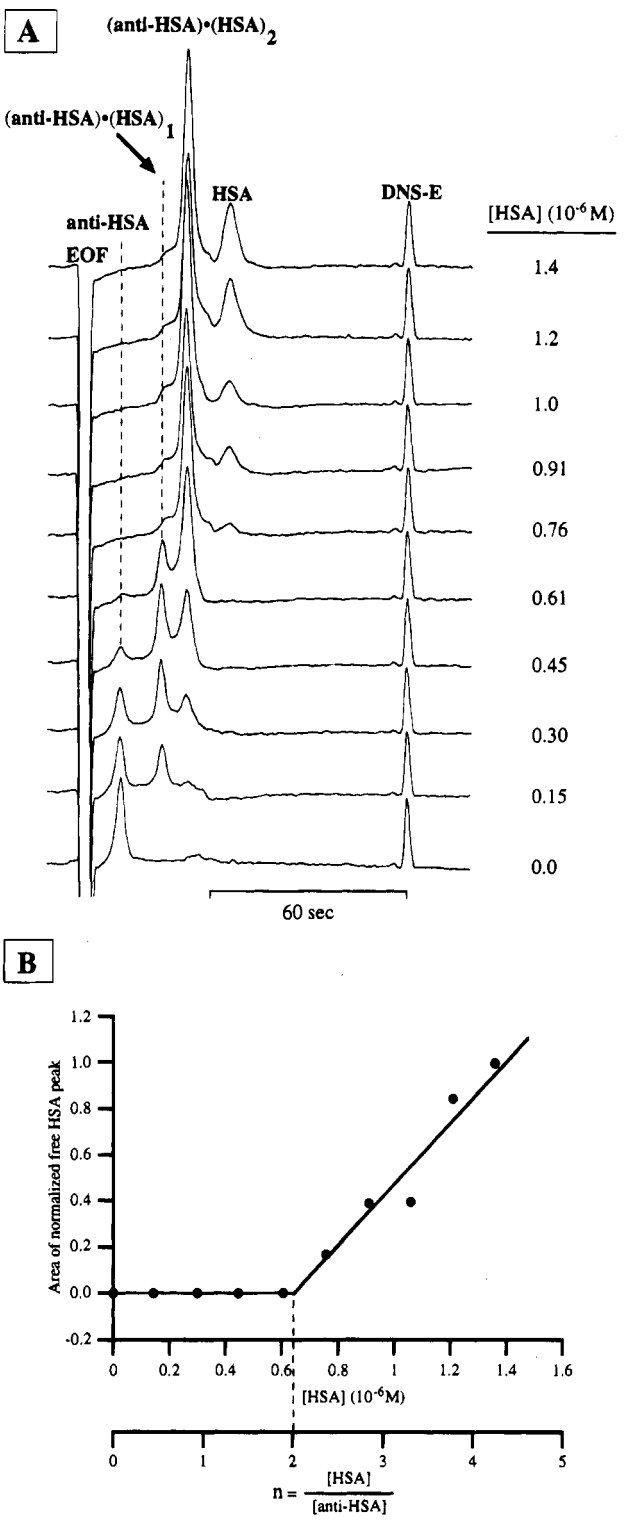


Figure 6. (A) Determination of the binding stoichiometry of human serum albumin (HSA) to its mouse monoclonal IgG antibody (anti-HSA, 0.33 μM) using ACE in tris-glycine buffer (pH 8.7)-200 nm as the detection wavelength. DNS-E was dansylglutamic acid used as an internal standard. (B) A plot of the concentration of free ligand vs the ratio of [HSA]/[anti-HSA] gives a sharp break at the stoichiometric point (2.0). (Data from Walsh, C. T., et al.; ref 8.)

R_L and L_R Experiments with Vancomycin (R) and D-Ala-D-Ala Peptides (L). We have also examined the use of ACE in studying binding interactions involving receptors with low molecular weight, using vancomycin as an example.[4] We have carried out two complementary types of ACE experiments with van-

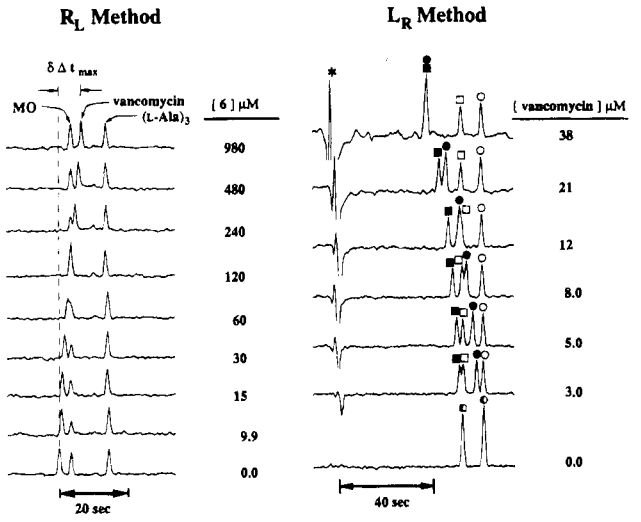
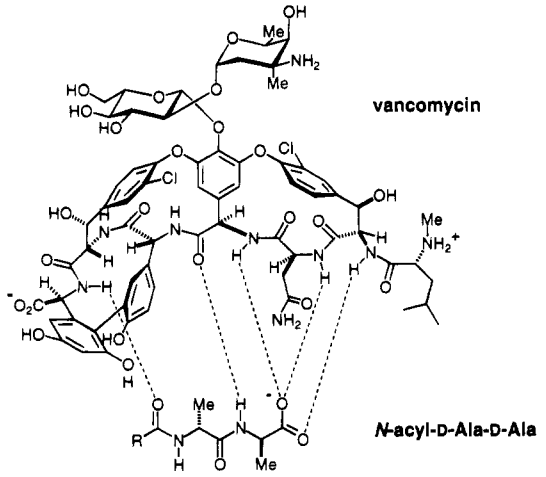


Figure 7. R_L Method: ACE of vancomycin in 10 mM sodium phosphate buffer (pH 7.1) containing various concentrations of *N*-acetyl-D-Ala-D-Ala, **6**. The neutral marker mesityl oxide (MO) and the tripeptide L-Ala-L-Ala-L-Ala were used as internal standards. L_R Method: ACE of *N*-Fmoc-Gly-D-Ala-D-Ala (●), *N*-Fmoc-Gly-D-Ala-D-Ala-D-Ala (■), *N*-Fmoc-Gly-L-Ala-L-Ala (○), and *N*-Fmoc-Gly-L-Ala-L-Ala-L-Ala (□) in 20 mM sodium phosphate buffer (pH 7.5) containing various concentrations of vancomycin. The asterisk (*) indicates the position of the peak for unidentified neutral species carried through the capillary by electroosmotic flow.

comycin (R) and peptidyl ligands (L = *N*-acetyl-D-Ala-D-Ala, **6**) (Figure 7).



The "R_L" Experiment. In this type of experiment, we observed the change in the mobility of the receptor vancomycin, μ_R, as we varied the concentration of ligand **6** in the buffer. This change in mobility of vancomycin was due primarily to the change in charge (from partial positive to partial negative) on binding the negatively charged peptide present in the electrophoresis buffer. Figure 7 shows representative electropherograms.

The "L_R" Experiment. In a second, complementary, type of experiment, we observed the mobility of the ligands, μ_L, as we varied the concentration of vancomycin included in the electrophoresis buffer (Figure 7). This change in mobility of the ligand was due primarily to the change in hydrodynamic radius and drag on binding the receptor.

The extreme economy with which ACE uses receptors and ligands permits both methods (R_L and L_R; especially the latter, where the receptor is used as a component of the buffer) to be practical. Using the principle of competitive binding, we have also described a rapid assay, by ACE, for use in identifying tight-binding ligands for vancomycin in mixtures of equimolar ligand candidates.[4]

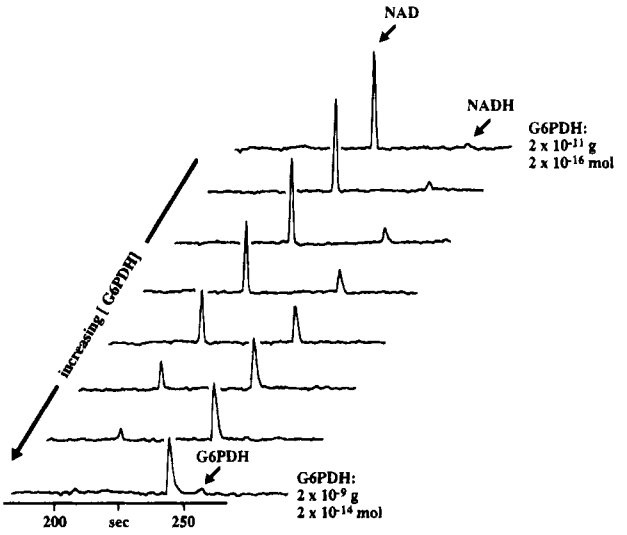


Figure 8. Sequential introduction of plugs of enzyme G6PDH, buffer, and substrate NAD (200 μM) followed by electrophoresis in buffer containing Glc-6-P (200 μM) results in enzyme-catalyzed conversion of the substrate to product as the substrate-containing plug migrates through the enzyme-containing plug. The amount of G6PDH in the enzyme plug was serially diluted by a factor of 2 in going from one electropherogram to the next.

The Capillary as a Microreactor

Regnier,[14] we,[5] and others have demonstrated that enzyme-catalyzed reactions take place in an electrophoresis capillary under nondenaturing conditions, and convert substrates to products. In thess experiments, enzymes and substrates are injected as separate plugs into the capillary (a procedure we call a "plug–plug reaction") or the substrate is included in the electrophoresis buffer (a "continuous reaction"). We have demonstrated the oxidation of glucose 6-phosphate (Glc-6-P) to 6-phosphogluconate using glucose-6-phosphate dehydrogenase (G6PDH) with NAD as a model system.

Plug–Plug Reaction. In the plug–plug reaction (Figure 8), samples of G6PDH and of NAD were injected separately onto a capillary and subjected to electrophoresis. The relative order and timing of injection onto the capillary are important to ensure contact between G6PDH and NAD during electrophoresis.

Continuous Reaction. In a second protocol (Figure 9), a sample of G6PDH was injected onto a capillary that had been equilibrated with a buffer containing NAD. This protocol is the extension of the plug–plug reaction to the reaction of a plug of G6PDH with a continuous stream of NAD (essentially an indefinitely long plug). The response of the electropherograms correlates with the amount of G6PDH present in the plug and with the duration of contact between G6PDH and NAD. This type of experiment allows amplification of enzymatic activity in the plug.

We observed both enzyme catalysis and the effect of ligand binding on the electrophoretic mobility of

Table 1. Studies on Binding of Ligands to Receptors Using Affinity Capillary Electrophoresis

example	ref
A. protein–protein interactions	
human serum albumin (HSA) and anti-HSA	8
human growth hormone (hGH) and anti-hGH (or its fragment)	24
B. protein–DNA interactions	
*Eco*R1 and oligonucleotide; peptide and oligonucleotide	25
C. protein–peptide interactions	
SH3 domain and proline-rich peptides	7
D. protein–drug interactions	
bovine carbonic anhydrases (CA) and arylsulfonamides; glucose-6-phosphate dehydrohenase (G6PDH) and $NADP^+$; G6PDH and NADPH; IgG_{2b} and 2,4-DNP	1, 2, 6, 23
bovine serum albumin (BSA), bacterial cellulase and tryptopha, benzoin, pindolol promethazine, warfarin	26
HSA and kynurenine, tryptophan, 3-indole lactic acid, 2,3-benzoyltartaric acid, (2,4-dinitrophenyl)glutamate	27
cellulase and β-blockers	28
BSA and warfarin	29
BSA and leucovorin	30
E. protein–metal ion interactions	
calmodulin, parvalbumin, thermolysin, and Ca(II); carbonic anhydrase, thermolysin, and Zn(II)	1, 31, 32
C-reactive protein and Ca(II)	25, 33
F. protein–carbohydrate interactions	
Tetragonolobus purpureas lectin and fucose 1-phosphate	34
β-galactose-binding lectin and lactobionic acid	35
concanavalin A–rhodamine-labeled mannoside	36
G. peptide–peptide interactions	
vancomycin and D-Ala-D-Ala-containing peptides	3, 4, 37
H. peptide–carbohydrate interactions	
synthetic peptide and anionic carbohydrates	38
I. peptide–dye interactions	
synthetic peptide and Congo Red	39
J. carbohydrate–drug interactions	
methyl-β-cyclodextrin and propranolol	40
K. oligonucleotide–oligonucleotide interactions	
$d(A)_n/d(T)_n$	41

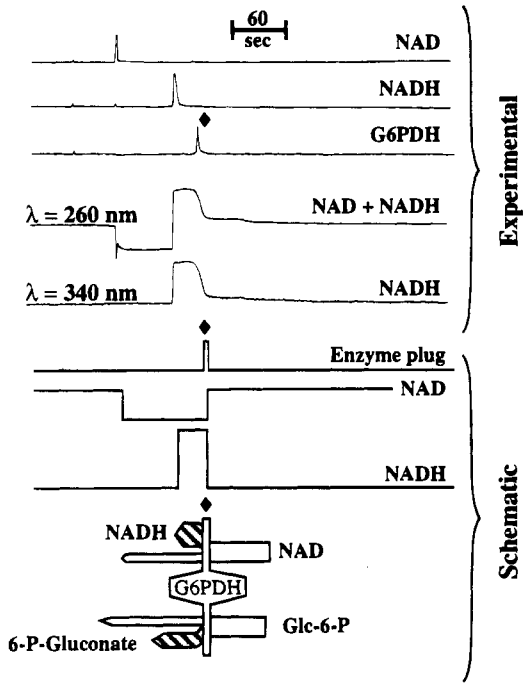


Figure 9. The relative migration times of G6PDH, NAD, and NADH (observed at 200 nm) serve as reference for the remainder of the figure. The experimental electropherograms were obtained at 260 and 340 nm with a plug of G6PDH migrating in a buffer containing NAD (200 μM) and Glc-6-P (200 μM). The schematic shows the analysis of the experimental electropherograms in terms of consumption of NAD and generation of NADH. The schematic section also shows the analysis of the experimental electropherograms in terms of the mobilities of the cofactors relative to the plug of enzyme. The arrows indicate substrate flowing through the plug in the direction shown; the narrowed width of the arrow coming out of the plug indicates reduction in the concentration of the substrate. The hatched arrows indicate the flow of the products in the direction indicated. The putative time of arrival of the enzyme at the detector in the presence of the substrates is represented by ◆.

G6PDH during these experiments. Potential applications of these microreactor techniques include analyzing samples for enzymatic activities or concentrations of substrates, evaluating molecules as substrates for enzyme-catalyzed reactions, examining mixtures of proteins for activity against a particular substrate, and studying enzyme–inhibitor interactions.

Conclusion

How General Is ACE? CE offers rapid analysis of small amounts of water soluble, charged samples with high resolution; ACE allows these advantages to be applied in studying binding interactions (Table 1). ACE can be valuable in determining both binding constants and binding stoichiometries of receptor–ligand interactions, in estimating kinetic constants for

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association and dissociation, and in screening tight-binding ligands from mixtures of compounds. It requires only small amounts of samples (nanoliter injection volumes, picograms of proteins). It does not involve radioactive materials and chemically immobilized ligands, and it does not require changes in absorption or emission spectra upon binding.

ACE can simultaneously measure multiple binding constants from samples of protein isozymes or various ligands; they need not be pure individually. The capillary can also serve as a microreactor to study enzyme-catalyzed reactions. It is possible to detect, using enzymatic amplification, 10 amol (attomol) (10^{-18} mol) of G6PDH.[5,14]

The capability to modify the charge of the protein without significantly modifying its hydrodynamic drag allows ACE to be used to estimate the effective charge of proteins without knowledge of their sequences and structures.

The major current limitation to ACE is the tendency for proteins with high molecular weight and/or high p*I* to adsorb on the walls of uncoated capillaries. Addition of zwitterions and salts to buffers,[42] use of coated capillaries,[43-45] and correction for changes in EOF decrease (but do not always eliminate) the influence of this adsorption.

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