with the T46N showing decreased affinity for pyrimethamine.

In order to probe perturbations of the more physiologically relevant E·I·NH complex, the K_i values were determined for the inhibitor with the mutants. In only one case does the cofactor impart a synergistic decrease in the binding affinity for the inhibitor. Although the K_i of pyrimethamine for the T46S mutant is a factor of 3.5 greater than the K_d, it still remains lower than the K_i for the wild-type enzyme. Compared to T46S, the T46N mutant exhibits a modest 4-fold increase in the K_i which corresponds to the increase observed for the S108N mutant over wild-type malarial enzyme. Overall the malarial and bacterial DHFRs respond similarly to active-site perturbation with respect to pyrimethamine binding.

Conclusions. Although, significant perturbations by the mutant DHFRs are observed for individual rate constants along the kinetic pathway, their impact on the steady-state parameters is minimized by their compensatory nature. These results are consistent with the transferable nature of kinetic effects observed for other DHFR mutations.[21]

The ability of pyrimethamine to distinguish between the bacterial and malarial enzymes is largely due to the threonine γ-methyl, with little impact on the overall catalytic effectiveness of the enzyme by mutations at this site. Recent isolation of mildly resistant *P. fariciparum* isolates containing the S108T mutation are in agreement with these findings.[9] A reduction in affinity of the malarial enzyme by a factor of 10 or less, accurately reflected in this *E. coli* protein set, has been shown to confer physiologically relevant resistance to the parasite.[6] Consequently, in the absence of X-ray structural data for the malarial DHFR-thymidylate synthase binary enzyme, *E. coli* DHFR can serve as a useful model of the malarial enzyme. The parasites' strategy of using catalytically silent mutations in order to become less sensitive to pyrimethamine might be circumvented in the future by an appropriate antifolate design.

Acknowledgment. We thank Dr. Patricia Jennings, for preparing the mutant DNA, and Kaye Yarnell, for the expert typing of the manuscript.

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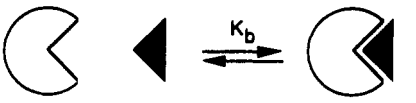
Use of Affinity Capillary Electrophoresis To Measure Binding Constants of Ligands to Proteins[1]

This paper outlines the use of affinity capillary electrophoresis[2,3] (ACE) as a technique for measuring binding constants of proteins for ligands. We illustrate this use with a model system comprising carbonic anhydrase B (CAB, EC 4.2.1.1, from bovine erythrocytes) and 4-alkylbenzenesulfonamides.[4] The principle of the method is illustrated schematically in eq 1.



(1)

Mass	M	m	M + m
Net Charge	Z	± z	Z ± z
Electrophoretic Mobility μ	~ Z / $M^{2/3}$		~ (Z ± z) / $(M + m)^{2/3}$

The electrophoretic mobility μ of a protein is related to its mass (M) and net charge (Z) by a relationship of the approximate form $\mu \sim Z/M^{2/3}$.[5] If the protein binds a charged ligand of relatively small mass, the change in μ due to the change in mass [from $M^{2/3}$ to $(M + m)^{2/3}$] is small relative to the change in μ due to the change in charge (from Z to $Z \pm z$). Thus, the protein–ligand complex will migrate at a different rate than the uncomplexed protein.[6] By measuring migration times (t) as a function of the concentration of charged ligand present in the buffer, it is possible to estimate K_b. These measurements are best carried out by measuring changes in t relative to another protein having a similar value of migration time

(1) This research was supported by the NSF through the M.I.T. Biotechnology Processing Engineering Center (Cooperative Agreement CDR-88-03014) and by the NIH (GM 39589 and GM 30367).

(2) Reviews of capillary electrophoresis of proteins: Mazzeo, J. R.; Krull, I. S. Coated Capillaries and Additives for the Separation of Proteins by Capillary Zone Electrophoresis and Capillary Isoelectric Focusing. *BioTechniques* **1991**, *10*, 638–645. Novotny, M. V.; Cobb, K. A.; Liu, J. Recent Advances in Capillary Electrophoresis of Proteins, Peptides, and Amino Acids. *Electrophoresis* **1990**, *11*, 735–749.

(3) Reviews of affinity electrophoresis (AE): Takeo, K. Affinity Electrophoresis. In *Advances in Electrophoresis*; Chrambach, A., Dunn, M. J., Radola, B. J., Eds.; VCH Publishers: New York, 1987; Vol. 1, pp 229–279. Shimura, K. Progress in Affinophoresis. *J. Chromatogr.* **1990**, *510*, 251–270. Horejsi, V.; Ticha, M. Qualitative and Quantitative Applications of Affinity Electrophoresis for the Study of Protein–Ligand Interactions: A Review. *J. Chromatogr.* **1986**, *376*, 49–67.

(4) Dodgson, S. J.; Tashian, R. E.; Gros, G.; Carter, N. D. *The Carbonic Anhydrases: Cellular Physiology and Molecular Genetics*; Plenum Press: New York, 1991.

(5) This expression is approximate. Other functional forms have been suggested: Rickard, E. C.; Strohl, M. M.; Nielsen, R. G. Correlation of Electrophoretic Mobilities From Capillary Electrophoresis With Physicochemical Properties of Proteins and Peptides. *Anal. Biochem.* **1991**, *197*, 197–207. Grossman, P. D.; Colburn, J. C.; Lauer, H. H. A Semiempirical Model for the Electrophoretic Mobilities of Peptides in Free-Solution Capillary Electrophoresis. *Anal. Biochem.* **1989**, *179*, 28–33. Deyl, Z.; Rohlicek, V.; Adam, M. Separation of Collagens by Capillary Electrophoresis. *J. Chromatogr.* **1989**, *480*, 371–378. Offord, R. E. Electrophoretic Mobilities of Peptides on Paper and Their Use in the Determination of Amide Groups. *Nature* **1966**, *211*, 591–593.

(6) If μ_b and t_b are the values of electrophoretic mobility and migration time of the protein–ligand complex, and μ_0 and t_0 are these values for free protein, eq i allows an estimate of the change in mobility or migration time for given values of Z and z.

$$(\mu_b - \mu_0)/\mu_0 = (t_b - t_0)/t_0 \approx [(z/Z) + 1][M/(M + m)]^{2/3} - 1 \approx z/Z \quad \text{(i)}$$

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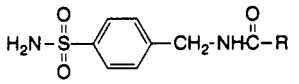
that does not bind the ligand. Equation 2 gives a convenient form for Scatchard analysis:

$$(\delta\Delta t/\delta\Delta t_{max})(1/[L]) = K_b - K_b(\delta\Delta t/\delta\Delta t_{max}) \quad (2)$$

here $\Delta t_{[L]}$ is the difference between the migration time of the protein of interest and the reference protein at concentration [L] of the charged ligand, $\delta\Delta t_{[L]} = \Delta t_{[L]} - \Delta t_{[L]=0}$, and $\delta\Delta t_{max}$ is the value of $\delta\Delta t_{[L]}$ at saturating concentrations of L.

We used CAB as a model protein with which to demonstrate this method and the aryl sulfonamides 1 and 2



1, R = $-(CH_2)_4CO_2^-$

2, R = $-(CH_2)_4CONHC[CH_2O(CH_2)_3SCH_2CO_2^-]_3$

3, R = $-CH(CH_3)_2$

as affinity ligands.[4] The samples of CAB also contained carbonic anhydrase A (CAA). We selected CAB for three reasons: it shows little adsorption on the walls of uncoated capillaries,[7] and is therefore a particularly convenient protein with which to work; it accepts a wide structural range of benzenesulfonamides as inhibitors; it is readily available and structurally well-characterized.

Figure 1 shows a representative series of electropherograms of CAB in buffer containing various concentrations of 1 and the Scatchard plot (eq 2) derived from these data. The appearance times[8] of horse heart myoglobin (HHM) and mesityl oxide (MO), used as internal standards in these experiments, were independent of the concentration of 1 and 2. Binding constants estimated by ACE (K_b = 0.48×10^6 M^{-1} for 1, 0.22×10^6 M^{-1} for 2) agree well with those obtained from a competitive fluorescence-based assay[9] (0.51×10^6 M^{-1} for 1, 0.14×10^6 M^{-1} for 2). CAA, a protein having binding constants very similar to those of CAB,[4] gives values of K_b estimated by ACE that are indistinguishable from those of CAB (Figure 1). The electrically neutral 3 used as the control ligand did not change the electrophoretic mobilities of CAB and CAA.[10]

Similar studies with proteins other than CAB have established that ACE is, in principle, a general method (although, in practice, it is limited to the subset of proteins that do not adsorb on the wall of the capillary). The mobility of calmodulin (from bovine testes) varies with the concentration of Ca^{2+}; analysis of this variation indicates $K_b = 0.47 \times 10^6$ M^{-1}; the literature values from equilibrium dialysis analysis are $0.1–1.0 \times 10^6$ M^{-1}.[11] Mobility of glucose-6-phosphate dehydrogenase (from *Leuconostoc mesenteroides*) varies with the concentration of both $NADP^+$ and NADPH; analysis of these variations gives K_b ($NADP^+$) = 0.03×10^6 M^{-1} (lit.[12] $K_b = 0.33 \times 10^6$ M^{-1} by fluorescence analysis) and K_b (NADPH) = 0.01×10^6 M^{-1} (lit.[12] $K_b = 0.04 \times 10^6$ M^{-1}). We have also examined a number of other proteins, including one antibody (MOPC 315). The results indicate that, although adsorption on the wall of uncoated capillary is a potential problem with all proteins, those having a p*I* significantly lower than the pH of the buffer are plausible candidates for analysis by

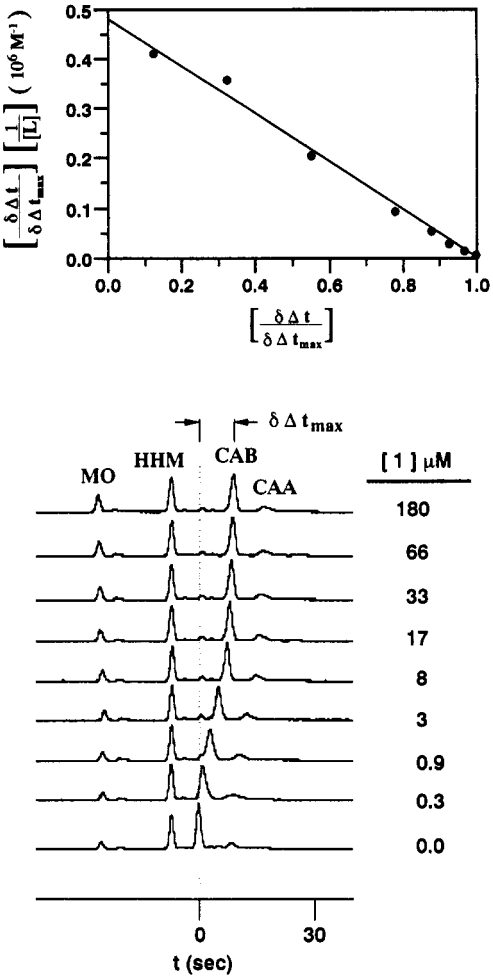


Figure 1. Affinity capillary electrophoresis (ACE) of bovine carbonic anhydrase B (CAB) in 0.192 M glycine–0.025 M tris buffer (pH 8.4) containing various concentrations of 1. The total analysis time in each experiment was ~5.5 min at 30 kV using a 70-cm (inlet to detector), 50-μm open quartz capillary. Horse heart myoglobin (HHM) and mesityl oxide (MO) were used as internal standards. The graph is a Scatchard plot of the data according to eq 2.

(7) Most proteins adsorb on the walls of uncoated capillaries.[2] This problem of protein adsorption can, in most cases, be solved by using some combination of coated capillaries and zwitterionic buffers. See: Towns, J. K.; Regnier, F. E. Capillary Electrophoretic Separation of Proteins Using Nonionic Surfactant Coatings. *Anal. Chem.* **1991**, *63*, 1126–1132. Bushey, M. M.; Jorgenson, J. W. Capillary Electrophoresis of Proteins in Buffers Containing High Concentrations of Zwitterionic Salts. *J. Chromatogr.* **1989**, *480*, 301–310.

(8) In capillary zone electrophoresis, migration times are determined by a combination of electrophoretic migration and electroosmotic flow (EOF). At pH values greater than 6, the EOF is sufficiently high to ensure a net migration toward the cathode for most analytes, regardless of their charge.

(9) The homogeneous assay followed modifications of the procedure of Chen, R.; Kernhan, J. Combination of Bovine Carbonic Anhydrase With a Fluorescent Sulfonamide. *J. Biol. Chem.* **1967**, *242*, 5813–5823.

(10) The binding constant of 3 to CA was 0.25×10^6 M^{-1}, measured by the fluorescence assay.

(11) Klee, C. B. Interaction of Calmodulin With Ca^{++} and Target Proteins. In "*Calmodulin*", *Molecular Aspects of Cellular Regulation*; Cohen, P., Klee, C. B., Eds.; Elsever: New York, 1988; pp 35–56. Kajiwara, H. Application of High-Performance Capillary Electrophoresis to the Analysis of Conformation and Interaction of Metal-Binding Proteins. *J. Chromatogr.* **1991**, *559*, 345–356.

(12) Grove, T. H.; Ishaque, A.; Levy, H. R. Glucose-6-Phosphate Dehydrogenase From *Leuconostoc mesenteroides*: Interaction of the Enzyme With Coenzymes and Coenzyme Analogs. *Arch. Biochem. Biophys.* **1976**, *177*, 307–316.

ACE using uncoated capillaries.[13]

Affinity capillary electrophoresis has six advantages as a method of determining binding constants. First, it requires only small quantities of protein and ligand: the complete series of experiments in Figure 1 (with five replicates of each experiment) consumed ~22 ng of carbonic anhydrases (CAB + CAA), and 1.3 mg of 1. Second, it does not require high purity for the protein or an accurate value of its concentration, since values of K_b are based on migration times, not peak areas. Measurement of K_b can, as a result of the high resolving power of capillary electrophoresis, be carried out on mixtures of proteins. Third, it is applicable simultaneously to several proteins in the same solution (for example, CAA and CAB in Figure 1). Fourth, it does not require the synthesis of radioactive or chromophoric ligands, although (as with 1 and 2) it will require the synthesis of a charged analog of a ligand if the ligand is itself electrically neutral. Fifth, it is capable of distinguishing forms of a protein that bind ligand from forms of the same protein that are denatured and do not bind ligand. Sixth, the commercial availability of automated instrumentation, and the high reproducibility of data, make it experimentally convenient.

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Supplementary Material Available: Experimental details for the preparation of 1–3 (5 pages). Ordering information is given on any current masthead page.

(13) Rapid equilibration of protein between the buffer and the wall of the capillary does not influence the value of K_b obtained by this method, unless the equilibration is influenced by the concentration of L or K_b is substantially different for adsorbed and soluble protein.

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Book Reviews

Compendium of Organic Synthetic Methods. By Michael B. Smith. John Wiley & Sons, Inc., New York. 1992. xix + 547 pp. 16 × 23.5 cm. ISBN 0-471-60713-4. $59.95.

This volume presents in abstract form the key functional group transformations, as well as many carbon bond forming reactions, described in the chemical literature from 1987 to 1989. New sections have been added to include the oxides of sulfur, nitrogen, and phosphorus, all of which are integral to the preparation of difunctional compounds. As with previous volumes, chemical transformations are classified first by the reacting functional group of the starting material and then by the functional group formed. The chemical reaction, reagents, yield, and stereochemistry are clearly shown. Indexes for monofunctional and difunctional compounds conveniently guide the user to specific tranformations as well as to specific reviews. Volume 7 also includes alphabetized headings and other minor format changes which add to its overall utility and ease of use. The book contains a complete author index.

This volume contains about 1250 examples of published methods for the preparation of monofunctional compounds, updating the 8100 in the first six volumes. In addition, 850 examples of the preparation of difunctional compounds and almost 100 reviews are included. All organic chemists will find this book a useful and convenient access to references to recently described synthetic methods.

Staff

Chemical Immunology. Volume 50. Integrins and ICAM-1 in Immune Responses. Edited by Nancy Hogg. S. Karger AG, Basel. 1991. viii + 168 pp. 17.5 × 24.5 cm. ISBN 3-8055-5429-X. $134.50.

The regulation of cell adhesion is a prerequisite for establishing and maintaining a normal host defense system. A broadly distributed family of transmembrane receptors, the integrins, are one of the main classes of molecules mediating cellular adhesion. This book provides a comprehensive overview of the structure and function of a number of integrins and also of intercellular adhesion molecule-1 (ICAM-1). Topics covered in the 10 chapters comprising this volume include the regulation of leukocyte integrin function, the role of VLA integrins in lymphocyte migration and recognition, common and ligand-specific integrin recognition mechanisms, and the role of $\alpha 4\beta 1$ in lymphocyte–endothelial cell interactions. The second part of the book details the importance of ICAM-1, an immunoglobulin and ligand for the leukocyte integrin LFA-1. Its role in inflammation and tumor development and its relevance as a receptor for rhinovirus and *Plasmodium falciparum*-infected erythrocytes are comprehensively reviewed.

The roles of integrins and ICAM-1 in the intricate network of the immune response have only recently been recognized. Thus, this volume will be vitally important to those concerned with modern immunology. Medicinal chemists researching in the areas of inflammation, tumor development, and rhinovirus infections will likewise find this book a valuable source of information.

Staff

Annual Reports in Medicinal Chemistry. Volume 26. Editor-in-Chief, James A. Bristol. Academic Press, Inc., San Diego, CA. 1991. xii + 369 pp. 17 × 25 cm. ISBN 0-12-040526-1 (alk. paper). $65.00.

Volume 26 continues in the format which is familiar to medicinal chemists. It is divided into seven sections which incorporate a total of 32 chapters. The major sections are as follows: (1) CNS Agents, (2) Cardiovascular and Pulmonary Agents, (3) Chemotherapeutic Agents, (4) Immunology, Endocrinology and Metabolic Diseases, (5) Topics in Biology, (6) Topics in Drug Design and Discovery, and (7) Trends and Perspectives. Each chapter updates, in 10 pages or less, including an exhaustive list of references, a significant area of research in medicinal chemistry of an emerging area of biological science anticipated to impact the future discovery and development of new therapeutic agents. In general, the chapters cover all aspects of the topic being addressed for 1990 or since the subject was last reviewed in *Annual Reports in Medicinal Chemistry*.

Annual updates are provided in many traditional areas. For the first time, in 1990, the chapter on antihypertensives has been replaced with three more mechanistically-related chapters: renin–angiotensin system, potassium channel activators, and vasoactive peptides. Several chapters address topics of great current interest, e.g. neuronal calcium channels, neurokinin antagonists,