Molecular Sieve Studies of Interacting Protein Systems

XI. ISOMERIZING SOLUTES*

HERBERT R. HALVORSON† AND GARY K. ACKERS

From the Department of Biochemistry, The University of Virginia, Charlottesville, Virginia 22901

SUMMARY

The behavior of isomerizing solutes on molecular sieve columns has been studied theoretically and experimentally. Solutions of the relevant transport equations predict the shape and movement of solute zones for such systems. An experimental investigation of one such system has been carried out: the reversible unfolding of hen egg lysozyme. Results of this study clearly illustrate the effects of finite reaction kinetics on the shapes of solute profiles. To elucidate the influence of chromatographic parameters on the solute profiles, numerical simulations have also been carried out based on the theoretical expressions derived. From these investigations some guidelines have been formulated to aid the design of optimal chromatographic systems for study of isomerizing proteins. In general the technique is best applied to systems with rate constants less than 3 hour⁻¹.

Interacting systems of macromolecules are involved in the self-assembly and self-regulation of biologically functional complexes. In spite of increasing awareness and interest in their properties, most macromolecular interactions have proved difficult to characterize, and new approaches to their analysis are needed. In a recent series of papers (1-12) the uses of molecular sieve techniques for analysis of self-associating solutes and ligand-binding reactions have been explored. In the present study we have investigated a different class of interacting systems. The reaction to be considered is an isomerization or reversible unfolding of a macromolecule. If the unfolding reaction fulfills the requirements of a two-state approximation (13), then it can be described by Equation 1

\[ A_1 \xrightleftharpoons[k_2]{k_1} A_2 \]  (1)

where \( A_1 \) and \( A_2 \) represent the two species (states) and \( k_1 \) and \( k_2 \) are the forward and reverse rate constants. Analyses of this problem have been presented previously for transport systems of the freely migrating type such as electrophoresis (14-19), sedimentation (20), and counter-current distribution (21). In this paper we develop the corresponding theory for transport of isomerizing solutes on molecular sieve columns and present an experimental investigation on the reversible unfolding of hen egg lysozyme which illustrates the effect of finite reaction kinetics on the shape of solute profiles. The unique feature of this technique is that differential transport of solute arises from the presence of a space-filling material, the stationary phase. Presence of the stationary phase requires the use of a new frame of reference and also generates unusual relationships between the parameters of solute molecular size, column dimensions, and the phenomena of translation and dispersion. To elucidate the effects of chromatographic parameters on the solute profiles, we have also carried out numerical simulations based on the theoretical expressions derived. Previous applications of gel chromatography to isomerizing systems (22, 23) have ignored the effect of reaction kinetics on the shape of the solute zone.

Recent work (24-26) has clearly demonstrated that the guanidine hydrochloride denaturation of lysozyme in acid is a fully reversible two-state process at temperatures below 30°. Under certain conditions employed in the present study the reaction rates are known to be very slow, on the order of hours, comparable to the duration of a chromatographic experiment (24). The magnitude of these rate constants has been confirmed by ultraviolet difference spectral measurements. In the studies to be described here denaturation was effected by decreasing the pH at constant guanidine hydrochloride concentration, thereby minimizing perturbations of the chromatographic system. (Variations in guanidine hydrochloride concentration may have a profound effect on the gel structure.) In the treatment which follows it is tacitly assumed that the gel column is inert to the means of effecting the reaction.

THEORY

The phenomenological theory of gel chromatography and the analytical application of this theory have been developed elsewhere (5-12, 27, 28). The treatment here will concentrate on a quantitative description of the shape of solute zones, based on solutions to the macroscopic continuity equations.

Definition of Variables

The notation of Ackers (27) will be used for the parameters of the chromatographic system. The bulk flow rate, \( F \), is

\[ \frac{k_1}{k_2} A_1 \xrightleftharpoons[k_2]{k_1} A_2 \]
assumed constant in distance, $x$, and time, $t$. The distribution volume, $V_t$, is the volume of the column accessible to the solute, and the partition cross-section, $\xi$, is the volume fraction which is accessible ($V_t/V$, where $V$ is the total volume of the column). The cross-sectional area of the column, $a$, is assumed constant; $\xi$ is the cross-sectional area accessible to the solute.

The coefficients of axial dispersion, $L$ and $L_F (=L/F)$, describe spreading of the solute zone (27, 28) due to processes of:

(a) nonuniform flow within the gel bed;
(b) diffusion along the column axis; and
(c) nonequilibrium exchange between mobile and stationary phases of the column. The forward and reverse rate constants, $k_1$ and $k_2$, have their usual significance.

In addition, it is necessary to define rate constants $k'_1$ and $k'_2$ in the "total column" frame of reference (10): $k'_1 = k_1/\xi a$, $k'_2 = k_2/\xi a$.

Transformation into this total column frame of reference accounts for the perturbation introduced by the presence of the stationary phase. The extent of this perturbation is directly proportional to the partition cross-section, the fraction of the column that is accessible to the solute. If $C$ is the normal bulk concentration (mass of solute per volume of solution, $Q/V$), then $C'$ is mass of solute per volume of column ($Q/V_t$ = ($Q/V$)/$V_t$) = $\xi C$). The concentration $C'$ is the quantity measured in direct scanning of gel columns (1, 3, 4). At equilibrium $k_1 C_1 = k_2 C_2$ and $k'_1 C'_1 = k'_2 C'_2$.

**Description of Solute Zones**

Within the assumption that $L$, $F$, $\xi$, and $a$ are independent of $x$, $t$, and $C$ ("ideal gel chromatography"), the phenomenological equations of continuity are

$$\frac{\partial C_1}{\partial t} = -L_1 \frac{\partial^2 C_1}{\partial x^2} + \frac{F}{\xi a} \frac{\partial C_1}{\partial x} = k'_1 C_1 + k'_2 C_2$$

$$\frac{\partial C_2}{\partial t} = -L_2 \frac{\partial^2 C_2}{\partial x^2} - \frac{F}{\xi a} \frac{\partial C_2}{\partial x} = k'_1 C_1 - k'_2 C_2$$

Continuity equations of this form show that the establishment of diffusion equilibrium is rapid within every local region of the column bed. Their validity for single solute systems has been verified experimentally (28, 29). One of the objectives of the present study is to compare solutions to the more complex system defined by Equations 2 with experimental data. There are three cases of interest which differ in the relation between rates of chemical equilibration and separation of species.

**Very Slow Reactions**—If the reaction is so slow (or equivalently, the separation process is so rapid) that equilibrium is not re-established during the course of the experiment, the equations are separable and one obtains a solute profile indistinguishable from that of a single solute species (28). The phenomenological parameters ($\xi$ and $L$), however, are averages of the species values as defined by Equation 5.

**Very Fast Reactions**—In the limit of a rapidly isomerizing solute is indistinguishable from that of a single solute species (28). The phenomenological parameters ($\xi$ and $L$), however, are averages of the species values as defined by Equation 5.

**Intermediate Rates**—The remaining case, with relaxation times for separation and reaction roughly equivalent, has no such simple solution. This is unfortunate, since many real systems would be expected to belong to this class. It is nevertheless possible to obtain numerical solutions for systems of this kind. By taking the Fourier transforms of the continuity equations and solving the two simultaneous first order ordinary differential equations which result, it is possible to get the Fourier transform of the total concentration. The boundary conditions are

$$C_1(x,0) = \delta(x)$$

$$k'_1 C_1(t=0) = k'_2 C_2$$

The latter equation states the assumption of initial chemical equilibrium. We introduce a linear velocity $v = F/\xi a$. Also, let the subscripts + and - on a quantity $z$ denote $z_1 + z_2$ and $z_1 - z_2$. Applying the complex Fourier transform

$$C_m(x, t) = \frac{\xi a}{\sqrt{\pi L}} \exp \left\{ -\left(x-Ft/\xi a\right)^2/4Lt \right\}$$

Solutions for other initial conditions can be found in Refs. 27 and 28. Equation 6 indicates that the chromatographic profile for a rapidly isomerizing solute is indistinguishable in form from that of a single solute species (28). The phenomenological parameters ($\xi$ and $L$), however, are averages of the species values as defined by Equation 5.

**Intermediate Rates**—The remaining case, with relaxation times for separation and reaction roughly equivalent, has no such simple solution. This is unfortunate, since many real systems would be expected to belong to this class. It is nevertheless possible to obtain numerical solutions for systems of this kind. By taking the Fourier transforms of the continuity equations and solving the two simultaneous first order ordinary differential equations which result, it is possible to get the Fourier transform of the total concentration. The boundary conditions are

$$C_1(x,0) = \delta(x)$$

$$k'_1 C_1(t=0) = k'_2 C_2$$

The latter equation states the assumption of initial chemical equilibrium. We introduce a linear velocity $v = F/\xi a$. Also, let the subscripts + and - on a quantity $z$ denote $z_1 + z_2$ and $z_1 - z_2$. Applying the complex Fourier transform

$$U_j(y,t) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} C_m e^{ivy} dx \ (j=1,2)$$

gives the ordinary differential equations:

$$\frac{dU_1}{dt} = -P_1 U_1 + k'_2 U_2$$

$$\frac{dU_2}{dt} = k'_1 U_1 - P_2 U_2$$

where $P = L y_0 - ivy + k'$. After solving for $U_1$ and $U_2$, their sum can be expressed as

$$U = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} C_m e^{-rv/2} \left\{ \cosh \left(v_2 Qt\right) + \frac{k'_2 + 4k'_2 k'\sqrt{Q}}{k'_2} \sinh \left(v_2 Qt\right) \right\}$$

where
The total concentration $C'_+\text{}$ is obtained by inverting the transform

$$C'_+ = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} U_+ e^{-iyx} dy$$

Analytic evaluation of this integral is a forbidding prospect, but it is possible to approximate the solution (18, 19) by expanding $U_+$ in a Taylor series about $y = 0$. Assuming the form $\exp \left( a_0 + ay + ay^2 + \cdots \right)$ for $U_+$ and truncating at the second power of $y$ gives

$$C'_+ = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} \frac{1}{2a} \exp \left\{-\frac{\phi^2}{4L^2t} \right\} dy$$

which is the equivalent of

$$C'_+ = \frac{1}{2a\sqrt{\pi L^2t}} \exp \left\{-\frac{\phi^2}{4L^2t} \right\}$$

where

$$\phi = x - \frac{Ft}{\bar{\xi} a}$$

and

$$L = \bar{\xi} + \frac{F\bar{\xi}^2K}{a^2(1 + K)^3\bar{\xi}^2k_2} \left[ 1 - O \left( \frac{1}{kt} \right) \right]$$

and $L$ and $\bar{\xi}$ have been defined before. As the time increases, the measured axial dispersion coefficient $L_{\text{exp}}$ approaches

$$L_{\text{exp}} = L_v + L_k$$

where $L_k$, the kinetic dispersion coefficient, is given by

$$L_k = \frac{F\bar{\xi}^2K}{\left[ a^2(1 + K)^3\bar{\xi}^2k_2 \right]}$$

The asymptotic solution (Equation 12) is also identical in form with that of a single solute species.

Converting Equation 12 to the concentration distribution of interest, $C_+$, is not a simple and straightforward matter, since rigorously the weight-average $\bar{\xi}$ will vary as one moves across the profile from regions rich in Species 1 to those rich in Species 2. We are interested in an asymptotic solution (wherein the species distribution across the profile should approach the equilibrium ratio) and furthermore the equilibrium is independent of total concentration. Therefore we have recourse to the relation

$$C_+ \approx C'_+ / \bar{\xi}$$

to give

$$C_+ = \left( 2\bar{\xi}a\sqrt{nL_vV} \right)^{-1} \exp \left\{ \frac{-(x-V/\bar{\xi}a)^2}{4L_vV} \right\}$$

An alternative determination of $C_+$ is from

$$C' + = C'_1 / \bar{\xi}_1 + C'_2 / \bar{\xi}_2$$

which does not contain the implication of a chemical equilibrium distribution across the solute zone. However, the individual transforms $U_1$ and $U_2$ do not lend themselves to the asymptotic approximation given above. Accordingly, the individual transforms were integrated numerically and combined for some of the simulations reported here.

EXPERIMENTAL PROCEDURES

Materials

No commercial gel proved to be sufficiently inert to the solvent perturbations or the solute (lysozyme) (or both), so 10% polyacrylamide (5% cross-linking) was prepared from specially purified materials. The gel was then ground in an Omni-Mixer (Sorvall), sieved (ASTM No. 50-No. 230; pore sizes: 297 and 62 μ, washed, and decanted several times.

The solvent was 3.9 M guanidine hydrochloride (Heico “Ultra High Purity”, Lot 217011) which was used without further purification. Concentration was determined by measuring the density and interpolating from the data of Kielley and Harrington (30). Aliquots were titrated to the desired pH with HCl.

The solutes were: glycylglycine (Mann 1719, Lot S2891), egg lysozyme (Sigma L-6876, Lot 70C-8110), and blue dextran (Pharmacia, Lot To4474), all used without further purification.

Methods

Chromatography—The columns were poured in 47.5-ml glass tubes (63 x 0.98 cm) after an initial equilibration with the solvent. Subsequent solvent changes were made by allowing the gel to equilibrate in situ. The columns were maintained at 20 ± 0.5°C with a water jacket (Lauda water bath, Brinkmann Thermoel). After traversing a flow cell, the eluate passed through a peristaltic pump (Varioperox, LKB Produkter A/B) and into a buret. Flow rates, determined from volume measurements, were constant within the accuracy of buret readings (~±0.02 ml per hour). The transmittance at 220 nm was monitored with a double beam spectrophotometer (Bausch and Lomb Spectronic 600). Elution profiles were recorded on a strip chart recorder (Beckman 93000) simultaneous with the taking of digital data at a rate of 120 points an hour (digital voltmeter-coupler: LSYP Inc; Teletype paper tape punch).

As the solvent meniscus entered the porous disk at the top of the column, 1 drop of equilibrated solution (containing 1 to 2 mg per ml of solute) was applied and the timer was started. One or two drops of solvent were applied as soon as the sample had entered the disk. When this had entered the disk, the space above the gel was rinsed and filled with solvent. The solvent reservoir was then connected, using a pressure head sufficient to keep a positive pressure on the peristaltic pump.

Computations were carried out with a HP 2114A digital computer and a HP 9100A programmable calculator.

Optical Studies—As a check on the chromatographic results, difference absorbance measurements at 301 nm were carried out to obtain independent estimates of the rate constants for denaturation and renaturation. Experiments at pH 3.35 were carried out using a Cary 14 spectrophotometer, the others in a Beckman DU-2. Samples were equilibrated over night at pH 6.15 (native) and 2.46 (denatured). Acid or base was added with rapid mixing, and timed absorbance measurements
were recorded. Rate constants were calculated from resulting linear first order plots.

RESULTS

For elution chromatography Equation 15 is written

$$C = \left(2 \xi a \sqrt{\pi L V}\right)^{-1} \exp \left\{ -\left(\frac{v - V}{\zeta a^2 LV}\right)^2 / 4 L V \right\}$$  \hspace{1cm} (17)

This relationship was used to obtain experimental values of $\xi$ and $L_V$ using a two-parameter nonlinear least squares analysis of the elution profile. These values are summarized in Table I. Fig. 1 compares the data and the theoretical curve for a representative experiment and indicates that the use of Equation 17 is valid. Recalling that $\xi = \bar{V}/V$,

$$\ln \left(C \sqrt{V}\right) = -\ln \left(2 \xi a \sqrt{\pi L V}\right) - \frac{1}{4 \xi a^2 LV} \frac{(V - \bar{V})^2}{V}$$  \hspace{1cm} (18)

so a plot of $\ln \left(C \sqrt{V}\right)$ versus $(V - \bar{V})^2/V$ should be linear. This comparison is made in Fig. 2 (same data as in Fig. 1). Since discernible deviation occurs only when $C$ is less than 0.5% of the value at the peak, it can be concluded that Equation 17 accurately describes the shape of the profile.

In Figs. 3 and 4 the data of Table I are presented as functions of pH. Although the values of $L_{\text{exp}}$ approximate a sigmoid transition curve with substantial base-line slope the values of $L_{\text{exp}}$ clearly describe a different function. This difference between the transition curves for the two parameters is an unequivocal demonstration of the existence of kinetic contributions to dispersion. Whereas the values of $\xi_{\text{exp}}$ represent the equilibrium distribution of species at each pH, the values of $L_{\text{exp}}$ also reflect the additional “kinetic dispersion.” The augmentation arises from the finite times (distributed randomly) in which a given molecular species will convert into the other. This produces a spreading of each species (and hence the total profile) which is unaccounted for by the equilibrium composition alone. Uncertainty in the desired parameters to be obtained from these data arises from uncertainty in construction of base-lines. After making a somewhat arbitrary extrapolation of the base-lines, values of $K$, $k_1$, and $k_2$ were derived from

$$K = \left(\xi_1 - \xi_{\text{exp}}\right) / \left(\xi_{\text{exp}} - \xi_2\right)$$

$$L_V = \left(\xi_1 L_{\text{exp}} + K \xi_2 L_{\text{exp}}\right) / \left(\xi_1 + K \xi_2\right)$$

$$L_k = L_{\text{exp}} - L_V$$

$$k_2 = F \left[\xi_1 - \xi_2\right]^2 K / \left[\alpha^2 (1 + K)^3 \xi_{\text{exp}} L_k\right]$$  \hspace{1cm} (19)

$$k_1 = K k_2$$

In calculating these quantities, estimates of the individual species parameters were obtained from the extrapolated base-lines at the appropriate pH. These values are summarized in Table II, along with results of the ultraviolet difference measurements.

DISCUSSION

This study has been directed toward three aspects of isomerization in gel chromatography: (a) conformity of profile shapes for isomerizing solutes to solutions of the proposed continuity equations; (b) the qualitative influence of reaction kinetics on solute profiles, to assess the feasibility of detecting such systems; and (c) an attempt to determine how much quantitative information on the kinetics can be derived.

Exploring the effects on the solute profile of varying system parameters is a venture best accomplished through numerical simulations. Simulated elution profiles offer the advantage of displaying the effects of varying precisely known parameters at will and over ranges that are not always experimentally accessible. Since the solutions are based on the ideal formulation of gel chromatography, they also provide a means of estimating the contributions of nonideality to the experimental profiles.

The simulations shown in Fig. 5 demonstrate the perturbation introduced by the inclusion of kinetic effects. Curve 1 is the profile which would occur in the absence of any reaction; Curve 2 would result if the reaction were infinitely rapid (instantaneous equilibrium). Curve 3 was obtained by applying
Equation 17. The discrete points were obtained by evaluating the individual Fourier integrals and using Equation 16. Among the features to be noted is the agreement between the two simulations which incorporate the kinetics of the reaction—all of the deviations can be attributed to accumulated truncation error in the numerical integrations. This provides additional validation of the treatment which led to applying Equation 17 to this system and further implies that the perturbation arising from nonequilibrium values of $\xi$ is slight. The second observation cuts both ways: despite the pronounced and clearly measurable difference between Curves 2 and 3, Curve 3 by itself hardly suggests the existence of an interacting (isomerizing) system. Even though the discrepancy is maximal at the equivalence point of the transition as shown here, quantitative information on the expected shape is needed to detect the kinetic effect. The profile still looks “reasonable” when $k$ is quite small, which is another way of saying that mixtures of similar compounds are difficult to resolve. The effect of varying $(\xi_1 - \xi_2)$, while holding other parameters constant, is shown in Fig. 6. Although there is greater sensitivity to this parameter, only when it approaches the physically unrealistic value of 0.2 does the elution profile take on an obviously bimodal shape. As expected, the maxima lie closer together than those appropriate to the individual species. These simulations illustrate the expected result that chromatographic detection of an isomerizing solute must come from changes in the position of the solute zone. This also applies to the problem of identification, for reversible association and all irreversible reactions must be ruled out. Once the end points of the transition have been well defined, detailed analysis can proceed based on changes in the shape of the solute zone. As is always true in such studies, one must have reliable values of the parameters for the individual species over the whole transition region. It is equally true that this information is not attainable. Judicious choice of the experimental variable and the chromatographic system would minimize the base-line slope shown in Figs. 3 and 4 and hence minimize the error in the derived results. It is most important that the internal volume of the column, the volume into which partitioning occurs, should remain constant over the range of the experi-

<table>
<thead>
<tr>
<th>$\text{pH}$</th>
<th>$F$</th>
<th>$\xi$</th>
<th>$L_v$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.65</td>
<td>8.915</td>
<td>0.566</td>
<td>0.547</td>
</tr>
<tr>
<td>4.60</td>
<td>8.894</td>
<td>0.568</td>
<td>0.549</td>
</tr>
<tr>
<td>3.55</td>
<td>8.909</td>
<td>0.567</td>
<td>0.549</td>
</tr>
<tr>
<td>3.25</td>
<td>8.899</td>
<td>0.569</td>
<td>0.585</td>
</tr>
<tr>
<td>2.80</td>
<td>8.902</td>
<td>0.559</td>
<td>0.435</td>
</tr>
<tr>
<td>2.50</td>
<td>8.923</td>
<td>0.540</td>
<td>0.418</td>
</tr>
</tbody>
</table>

Fig. 3. Partition cross-section ($\xi_{\text{exp}}$) as a function of $\text{pH}$. The base-lines have been extrapolated through the transition region to provide estimates of $\xi_1$ and $\xi_2$ over the $\text{pH}$ range.

Fig. 4. Axial dispersion coefficient ($L_v$) as a function of $\text{pH}$. Base-lines are extrapolated to provide estimates of $L_v$ and $L_{v2}$. The dashed line (---) corresponds to $L_v$, the equilibrium value defined in Equation 5. Kinetic dispersion $L_k$ is the difference between the solid line and the dashed line.
TABLE II

Kinetic analysis

A. Chromatographic results

<table>
<thead>
<tr>
<th>pH</th>
<th>K_exp</th>
<th>Calc</th>
<th>Lk</th>
<th>k1</th>
<th>k2</th>
<th>s^4</th>
<th>s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cm^-1</td>
<td>s^-1</td>
<td>s^-1</td>
<td>s^-1</td>
<td>s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.55</td>
<td>0.50</td>
<td>0.70</td>
<td>0.125</td>
<td>0.16 × 10^-4</td>
<td>1.08 × 10^-4</td>
<td>5900</td>
<td></td>
</tr>
<tr>
<td>3.25</td>
<td>0.69</td>
<td>2.17</td>
<td>0.004</td>
<td>2.53 × 10^-3</td>
<td>3.80 × 10^-4</td>
<td>343</td>
<td></td>
</tr>
</tbody>
</table>

B. Ultraviolet difference measurements

<table>
<thead>
<tr>
<th>pH</th>
<th>k_vap</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>s^-1</td>
<td>s</td>
</tr>
<tr>
<td>3.35</td>
<td>1.17 × 10^-9</td>
<td>853</td>
</tr>
<tr>
<td>3.90</td>
<td>3.77 × 10^-4</td>
<td>2652</td>
</tr>
<tr>
<td>4.84</td>
<td>4.84 × 10^-4</td>
<td>2070</td>
</tr>
<tr>
<td>3.42</td>
<td>1.56 × 10^-2</td>
<td>1590</td>
</tr>
<tr>
<td>3.94</td>
<td>1.20</td>
<td>734</td>
</tr>
<tr>
<td>7.94</td>
<td>3.50 × 10^-3</td>
<td>324</td>
</tr>
</tbody>
</table>

* The relaxation time r has its usual significance as time required for extent of reaction to change to 1/e of its initial value.

The unfolding reaction of lysozyme in guanidine hydrochloride provides a 2-fold stringent test for the chromatographic analysis of isomerizing solutes. First, there is substantial nonideality in the behavior of the chromatographic system (nonconstant base-lines). Second, because of the four disulfide bonds in lysozyme the change in hydrodynamic volume is small. From measurements of intrinsic viscosity (24) we calculate Stokes radii of 18.1 Å and 23.6 Å for States 1 and 2—a change of only 5.5 Å. This contrasts with a value of 33.9 Å for the unfolded molecule with the disulfides reduced ("random chain"). Finally, it is worthwhile to point out that Equation 1 is the equivalent of

\[
A_1 + B \frac{k_1}{k_2} A_2
\]
where \( A_z \) is a ligand-protein complex or some other reaction product and \( B \) is present to such an excess that its concentration is effectively constant. To see the effect of the typical ligand-binding reaction by this technique, \( B \) must be the macromolecule, of course.

Acknowledgment—We thank Dr. Rex Lovrien (Department of Biochemistry, University of Minnesota, St. Paul, Minn.) for generous use of facilities for the ultraviolet difference spectral measurements.

REFERENCES

Molecular Sieve Studies of Interacting Protein Systems: XI. ISOMERIZING SOLUTES
Herbert R. Halvorson and Gary K. Ackers


Access the most updated version of this article at http://www.jbc.org/content/249/3/967

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/249/3/967.full.html#ref-list-1