Biophysical Chemistry 7 (1978) 367–373

ISOELECTRIC FOCUSING OF A DIMERIZING SOLUTE IN RAPID CHEMICAL EQUILIBRIUM: COMPARISON OF SIMULATION PROCEDURES*

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Received 31 May 1977

The approach to isoelectric focusing equilibrium of a rapidly dimerizing solute was simulated by two different computing procedures: a stationary-grid model developed by Cann and Stimpson and a distorted-grid technique derived from the method of Cox. The results given by the two models were virtually identical at all times during the approach to equilibrium. Of the two procedures, the distorted-grid method has an advantage in computing time, while the stationary-grid model is applicable to a broader range of transport experiments. The effect on the focusing experiment of varying the electric field was examined by distorted-grid simulations. When the field was increased, the equilibrium distribution sharpened somewhat and the peak of the concentration profile shifted toward the isoelectric position of the dimer. The rate of approach to equilibrium was approximately proportional to the field strength.

1. Introduction

Chemically interacting solutes behave in distinctive ways during transport experiments. The shape of the moving boundary produced by a particular interacing system during sedimentation, chromatography, or electrophoresis depends on the properties of the system — stoichiometry, component molecular weights, reaction rates, and equilibrium constants [1–4]. Computer models can predict the behavior of interacting solutes in transport experiments and can thus help identify chemically reacting systems that may be encountered experimentally.

During the past fifteen years, several simulation procedures have been developed that are capable of dealing with the transport of interacting systems of various kinds [5–8]. Each technique was devised in response to a specific problem, but, as the different procedures evolved independently, they have come to be interchangeable for many problems. It is often possible to choose among different computing procedures to simulate a particular transport experiment, and it is desirable to choose as intelligently as possible on the basis of precision and computing efficiency.

Since the alternative simulation procedures have been developed in different laboratories, they have been coded for different operating systems and have been applied, for the most part, to different problems. For that reason, the results and computing times given in the literature cannot be compared simply. The most obvious way to compare any two models is to use both of them to simulate the same process on the same computer. No such direct comparison has been reported.

An opportunity recently arose to make a direct comparison. Cann and Stimpson [9,10] have adapted the simulation technique of Cann and Goad [6,11] to describe the approach to isoelectric focusing equilibrium of chemically interacting solutes. Their imme-

* Supported in part by Research Grants HL 13909-25 from the National Heart and Lung Institute (J.R.C.) and GM22243-02 from the National Institute of General Medical Sciences (D.J.C.), National Institutes of Health, United States Public Health Service, and by the Kansas Agricultural Experiment Station. This publication is No. 674 from the Department of Biophysics and Genetics, University of Colorado Medical Center and No. 196-j from the Department of Biochemistry, Kansas Agricultural Experiment Station.

diate interest was in predicting certain experimental anomalies produced by interactions of isomerizing macromolecular solutes with the pH gradient or with specific components of the ampholyte system, but they also simulated the approach to focusing equilibrium of a dimerizing solute in rapid chemical equilibrium. In pursuing the latter problem, it was of interest to consider the effect of changing the electric field on the transport process and on the equilibrium distribution. For this purpose, it seemed that an alternative simulation procedure adapted from the model of Cox [8,12] might require less computing time. Such a program was developed and has been processed in the operating system used by Cann and Stimpson. A direct comparison between the two general kinds of simulation techniques is thus possible for the first time, and the result is reported here. The new model required less computing time; it was immediately used to examine the effect of field strength on a representative focusing experiment. These results are also described here.

2. Methods

The solutes of interest are in monomer-dimer equilibrium: $2A \rightleftarrows A_2$. The equilibrium is said to relax very rapidly after the total concentration changes, so that the monomer and dimer concentrations are related by an equilibrium constant at all times everywhere in the system [1]:

$$K_2 \equiv C_2/C_1^2 . \tag{1}$$

$$C_T = C_1 + C_2 = C_1 + K_2 C_1^2 , \tag{2}$$

where C_1 and C_2 are the weight concentrations of monomer and dimer and C_T is the total concentration.

It is assumed that the pK of one or more ionizable groups on the macromolecule is altered due to dimerization, thereby leading to a change in isoelectric point. Accordingly, hydrogen ions are involved in the dimerization reaction, and K_2 must be a function of hydrogen ion concentration. We assume the K_2 is an insensitive function of pH and can be considered constant in the region of the isoelectric point, given the shallow pH gradient alone the isoelectric focusing column.

The simulation procedure developed for isoelectric focusing by Cann and Stimpson has been reported elsewhere [9] and need not be described here in detail. The calculation begins by dividing the system into short segments by placing boundaries at regular intervals along the isoelectric focusing column. The initial solute distribution is defined by assigning a value to the mean concentration in each segment. Appropriate finite difference expressions are used to compute the flow of monomer and of dimer due to electrophoresis and diffusion across each boundary in the array during a short time interval. The new total concentration of solute is calculated in each segment, and the corresponding monomer and dimer concentrations are computed from the equilibrium expression. The calculation then proceeds by alternating rounds of simulated flow and reequilibration until, for all practical purposes, the focusing equilibrium distribution is reached. The method was derived from techniques originally developed to simulate electrophoresis and sedimentation. The principal differing feature of the model for isoelectric focusing is that the array of boundaries between segments is held stationary instead of being moved as a unit at a velocity related to the migration rate of the macromolecular solute [6,11]. The change is required by the fact that, in isoelectric focusing, the solute moves from both ends of the array toward the middle rather than moving in one direction from the top of the array toward the bottom. Because of the stationary array of boundaries, formulation of the numerical calculation must of necessity trade off computer time for accuracy. In what follows, the method of Cann and Stimpson will be called the stationary-grid technique.

An alternative model was developed for the present work from the technique used by Cox to simulate sedimentation in the ultracentifuge [8,12]. This model for isoelectric focusing will be more completely described since it has not been reported previously. For reasons that will become evident, the procedure will be referred to as the distorted-grid model. The calculation begins, as does the stationary-grid model, by dividing the system into segments by placing boundaries at equal intervals along the isoelectric focusing column. If boundaries 1 and n are the ends of the array, there are $n - 1$ segments; segment i lies between boundaries i and $i + 1$, and boundary i separates

segments $i-1$ and i. In what follows, $\bar{C}_i$ is the mean concentration in *segment* i, and C_i is the concentration at *boundary* i, $\bar{x}_i$ is the midpoint of *segment* i, and x_i is the position of *boundary* i. The initial concentration in each segment is specified. The most convenient initial distribution centers a broad region of uniform concentration in the segment array and places regions of zero concentration at each end. The simulation consists of alternate rounds of diffusion without electrophoresis and electrophoresis without diffusion. The dimerizing solute is treated as a single component with a concentration-dependent diffusion coefficient and electrophoretic mobility.

Difussion is described by a finite difference analog of Fick's second law that has been used for the ultracentrifuge [8], with the straightforward modifications required by the fact that the isoelectric focusing column is cylindrical or rectilinear rather than sector-shaped. For a rapidly equilibrating monomer-dimer system, diffusion across the boundary between two adjacent segments is governed by an average diffusion coefficient:

$$D = \frac{D_1 C_1 + 2D_2 C_2}{C_1 + 2C_2} \tag{3}$$

where subscripts 1 and 2 refer to monomer and dimer [8,13]. The local concentrations of monomer and dimer at the boundary are related to the total solute concentration by eq. (2), and so D is an unambiguous function of C_T. An array of concentration gradients g^* between segments is calculated and assigned to the points x^* equidistant from the midpoint of adjacent segments:

$$g_i^* = \frac{\bar{C}_i - \bar{C}_{i-1}}{\bar{x}_i - \bar{x}_{i-1}}, \quad \bar{x}_i = \frac{x_{i+1} + x_i}{2}, \quad x_i^* = \frac{\bar{x}_{i-1} + \bar{x}_i}{2} \tag{4}$$

These points do not necessarily coincide with the segment boundaries, since the array of boundaries is distorted by the electrophoresis routine as explained below. The gradients at the boundaries g_i are found by interpolation in the g^* array. The change in the solute concentration in segment i due to diffusion during a time interval Δt_D is

$$\Delta \bar{C}_i = \frac{\Delta t_D (D_{i+1} g_{i+1} - D_i g_i)}{x_{i+1} - x_i} \tag{5}$$

where D_i is the average diffusion coefficient [eq. (3)] appropriate for the total concentration at boundary i. The concentration array is corrected and the process is repeated n_D times, simulating diffusion for a time equal to $n_D \Delta t_D$.

The simulation program then proceeds to a round of electrophoresis without diffusion. The distinctive feature of this model is that the solute is not driven across the boundaries between adjacent segments. Instead, the boundaries are moved for a short time at the local weight average velocity of the macromolecular solute [8]. The boundaries do *not* pass and are *not* passed by any of the solute; whatever solute was initially in the segment between two adjacent boundaries is still in the segment at the end of the time interval. Since the boundaries usually move at different rates, the lengths of the segments change. The volumes change while the mass of solute remains constant, and so the concentrations in the segments change.

The simulations we have done are for a simple case in which the electrophoretic mobility is a linear function of pH and the pH gradient is also linear. In this case, it is particularly straightforward to compute the weight average velocity of the solute at each point in the electrophoresis column and so to find the distance each boundary should be moved during a short time interval Δt_e. At position x in the column, the local velocities of the monomer, v_1, and the dimer, v_2, are:

$$v_1 = k_1(x_1 - x), \quad v_2 = k_2(x_2 - x), \tag{6}$$

where x_1 and x_2 are the isoelectric positions of the monomer and dimer; k_1 and k_2 are the absolute velocity gradients and are directly proportional to the applied electric field strength. The local weight average velocity and so the rate at which the boundaries should be moved is:

$$\bar{v} = \frac{dx}{dt} = f_1 v_1 + f_2 v_2 = f_1 v_1 + (1 - f_1) v_2, \tag{7}$$

where $f_1 = C_1/C_T$ and $f_2 = C_2/C_T$ are the local weight fractions of monomer and dimer. Substituting eq. (6) into eq. (7) and rearranging:

$$dx/dt = a - bx, \tag{8}$$

$$a = f_1 k_1 x_1 + (1 - f_1) k_2 x_2, \quad b = f_1 k_1 + (1 - f_1) k_2.$$

Integrating eq. (8) from time t to $t + \Delta t_e$ gives:

$$\ln\left(\frac{a - bx'}{a - bx}\right) = -b\Delta t_e, \tag{9}$$

where x is the position of the boundary at time t and x' is the new position after the time interval Δt_e. Eq. (9) can be simplified to give:

$$x' = A + (x - A)B$$

$$A = \frac{f_1 k_1 x_1 + (1 - f_1) k_2 x_2}{f_1 k_1 + (1 - f_1) k_2}, \qquad (10)$$

$$B = \exp[-(f_1 k_1 + (1 - f_1)\Delta t_e] .$$

Notice that A and B are independent of position in the column. They depend only on the local weight fraction of monomer and are thus specified unambiguously by the total local concentration of the dimerizing solute.

A round of simulated electrophoresis begins by finding the total concentration at each boundary by interpolation between the segments on either side. The corresponding values of A and B are obtained and a new set of boundary positions is computed using eq. (10). The new concentration after the time interval Δt_e is:

$$\bar{C}_i' = \bar{C}_i \frac{x_{i+1} - x_i}{x_{i+1}' - x_i'} . \qquad (11)$$

The electrophoresis routine is applied to the new solute distribution and the procedure is repeated. One round of simulated electrophoresis consists of one or more successive applications (n_e) of the electrophoresis routine. The number of operations in each round of diffusion, n_D, and of electrophoresis, n_e, need not be the same. However, n_D, n_e, and the time intervals Δt_D and Δt_e must be selected so that $n_D \Delta t_D = n_e \Delta t_e$; that is, equal times are spent in diffusion without electrophoresis and electrophoresis without diffusion. The program then returns to the diffusion routine and continues with alternate applications of diffusion and electrophoresis until focusing equilibrium is reached.

Calculating the diffusion coefficient D or the electrophoresis parameters A and B appropriate for a particular total solute concentration is a moderately time-consuming operation using eqs. (2) and (3) or eqs. (2) and (10). The appropriate transport coefficients must be found at each boundary before each step in the calculation, and so a very long simulation of the kind described here may repeat the operation several hundred thousand times. The computing time required can be greatly reduced by a table search routine similar to the one used in simulating sedimentation in the ultracentrifuge [8]. Before the simulation begins, D, A, and B are calculated and stored for each of several hundred values of the total solute concentration, C_T. Then, before each step in the simulation, the value of D or of A and B appropriate for the concentration at each boundary is found by interpolation in the table. The table search can be made much more efficient than direct computation of the transport parameters, and the saving in computing time is very large.

Since the boundaries move inward toward the isoelectric positions near the middle of the array, the segments between them become progressively narrower If this process continued without limit, the segments would become very narrow, and unacceptable computing artifacts would result. For this reason, the array is inspected after each application of the electrophoresis routine; when any segment is found to have less than half the original segment length, it is merged with the shorter of the two segments on either side. The continuous elimination of excessively narrow segments produces another difficulty. There are progressively fewer segments, and, as the entire system is compressed the concentration profile is no longer adequately isolated from the ends of the array. To eliminate this problem, empty segments are added to the top and bottom of the array as needed to provide at least n_D empty segments at each end before each application of the diffusion routine.

It should be noticed that the inward migration of boundaries and the elimination of compressed segments continues indefinitely and does not stop when focusing equilibrium is reached. The solute distribution becomes constant when the rate at which solute is carried inward by the moving boundaries is balanced everywhere by diffusion outward across the boundaries.

3. Results

The dimerizing solute used in all of the simulated experiments was given a weight-scale association constant [eq. (1)] of 1.0 ml/mg; with this association constant, the concentrations of monomer and dimer are equal at a total concentration of 2.0 mg/ml. The beginning concentration array was made 2.0 cm long. The initial concentration was made 1.0 mg/ml between $x = 0.54$ cm and $x = 1.44$ cm and was set to zero

between $x = 0$ and $x = 0.54$ cm and between $x = 1.44$ cm and $x = 2.00$ cm. The isoelectric positions of the dimer and the monomer were placed at 0.75 cm and 1.25 cm respectively. The diffusion coefficient of the monomer was 5.1×10^{-7} cm^2/s; that of the dimer was 3.6×10^{-7} cm^2/s.

Comparison of simulation procedures: In the case used to compare the two simulation procedures, the absolute velocity gradients, k_1 and k_2 in eq. (6), were both set equal to 1.0×10^{-4} s^{-1}. The calculations were allowed to proceed for a simulated time of 200 000 s. The progress of the simulations toward focusing equilibrium was monitored by computing the centroid and the variance of the solute distribution at frequent intervals. The mass of solute per unit cross-section in segment i is:

$$m_i = \bar{C}_i(x_{i+1} - x_i) . \tag{12}$$

The centroid $\bar{X}$ of the distribution is:

$$\bar{X} = \sum_{i=1}^{n} m_i \bar{x}_i / \sum_{i=1}^{n} m_i \tag{13}$$

and the variance is:

$$\sigma^2 = \sum_{i=1}^{n} m_i(\bar{x}_i - \bar{X})^2 / \sum_{i=1}^{n} m_i = \sum m_i \bar{x}_i^2 / \sum m_i - \bar{X}^2 . \tag{14}$$

The system was taken to be at focusing equilibrium when there was no further appreciable change in $\bar{X}$ or σ^2.

The results obtained by the stationary-grid and the distorted-grid procedures were compared by inspecting the concentration profiles and by comparing the calculated centroids and variances. By every measure the two procedures gave virtually identical results at all times during the approach to focusing equilibrium. The variance reached its final value considerably sooner then did the centroid; that is, the solute band continued to shift slowly after becoming as sharp as it would be at equilibrium, as predicted analytically for isomerization reactions by eqs. (5) and (6) of ref. [9]. Neither the variance nor the centroid changed significantly after 90 000 s. Fig. 1A shows the calculated solute distribution after 200 000 s. The points from the distorted-grid model fall precisely on the solid line given by the stationary grid simulation. The relative

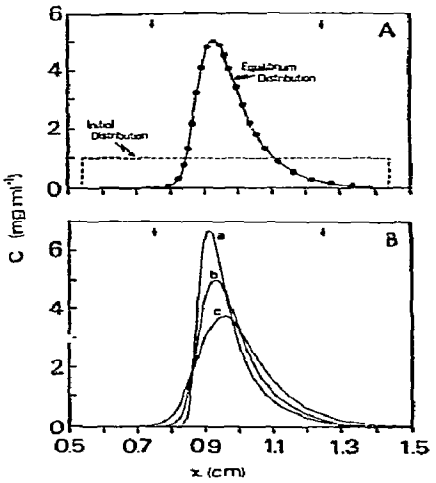

Fig. 1. Simulated isoelectric focusing of a dimerizing solute. Equilibrium constant: $K = 1.0$ ml/mg; diffusion coefficients: $D_1 = 5.1 \times 10^{-7}$ cm^2/s and $D_2 = 3.6 \times 10^{-7}$ cm^2/s; isoelectric positions: $x_1 = 1.25$ cm and $x_2 = 0.75$ cm. A. Comparison of procedures: solid curve, stationary-grid model; points, distorted-grid model; absolute velocity gradients: $k_1 = k_2 = 1.0 \times 10^{-4}$ s^{-1}; time = 200 000 s. B. Effect of field: Curve a. $k_1 = k_2 = 2.0 \times 10^{-4}$ s^{-1}, time 45 000 s; Curve b. $k_1 = k_2 = 1.0 \times 10^{-4}$ s^{-1}, time = 200 000 s; Curve c. $k_1 = k_2 = 0.5 \times 10^{-4}$ s^{-1}, time = 200 000 s.

breadth and skewing of the distribution are the features that distinguish a rapidly equilibrating monomer-dimer system from a single, non-interacting component. No subsidiary peaks or shoulders are expected in such a case.

Although the two simulation techniques produced virtually identical solute distributions, their performance differed in two significant ways. First, the distorted-grid technique was much faster. In the Control Data 6400 system used for both computations, the distorted-grid calculation ran in about five minutes while the stationary-grid model required approximately an hour.

The other difference between the two calculations arose from the different structures of the two models [8,11]. The stationary-grid model computes the combined flow due to diffusion and electrophoresis at each step. As the equilibrium distribution is approached, the flow across each boundary decreases smoothly to zero. The mass of solute in each segment

and thus the centroid and variance of the distribution remain absolutely constant in time to seven decimal places once focusing equilibrium has been reached as judged by this criterion. The distorted-grid model, on the other hand, alternates diffusion outward and electrophoresis inward, and these two opposing processes persist indefinitely. The array of boundaries continues to move inward and compressed segments are merged from time to time even after equilibrium is achieved. The centroid and variance [eqs. (13) and (14)] are computed, in effect, by interpolation in the continuously changing array of boundaries, and so these quantities fluctuate within a narrow range around the correct equilibrium values. The maximum range of fluctuation observed was typically $\pm 0.5\,\mu$, and so the effect causes no practical difficulty.

Effect of varying field: The distorted-grid model was used to simulate the effect of changing the electric field strength on the distribution of a dimerizing solute at isoelectric focusing equilibrium and on the rate of approach to equilibrium. In these calculations, all of the input variables describing the solute and the experimental conditions were the same as in the initial experiment, except for the absolute velocity gradients k_1 and k_2 [eq. (6)], which were given the values $k_1 = k_2 = 2.0 \times 10^{-4}\ s^{-1}$ in one simulation and $k_1 = k_2 = 0.5 \times 10^{-4}\ s^{-1}$ in another. These changes from the previous values $k_1 = k_2 = 1.0 \times 10^{-4}\ s^{-1}$ had the effect of doubling and halving the electric field while keeping the pH gradient constant.

The equilibrium distributions obtained at three different electric field strengths are shown in fig. 1B. An additional experiment done with $k_1 = k_2 = 1.5 \times 10^{-4}\ s^{-1}$ gave a distribution intermediate between curves a and b in the figure. All of the profiles were strongly skewed. Increasing the field shifted the peak of the distribution toward the isoelectric position of the dimer, because the concentration profile sharpened as the field was increased, which, in turn, resulted by mass action in an overall increase in the fraction of dimer through the profile. The sharpening was not as pronounced, perhaps, as might have been expected; doubling the field increased the concentration at the maximum by about a third in these particular experiments. All of the profiles — including the one not shown with $k_1 = k_2 = 1.5 \times 10^{-4}\ s^{-1}$ — shared a common point at $x = 0.86$ cm. The significance of this result is poorly understood, but analogous stationary points have been seen in previous simulations of the transport of associating solutes [14].

For $k \times 10^4 = 2.0$, 1.5, 1.0 and 0.5, the times required to reach focusing equilibrium were approximately 36 000, 60 000, 90 000 and 175 000 seconds, respectively. These are relatively crude estimates, since the small fluctuations of the calculated centroid around the equilibrium position made it difficult to judge precisely when equilibrium had been achieved. The time to each equilibrium appeared to be roughly inversely proportional to the electric field strength. The result differs from the behavior of solutes undergoing ampholyte- or pH-induced isomerization; in those cases, lowering the field strength increases the rate of the second, diffusion-controlled kinetic phase of approach to equilibrium [9,10].

4. Discussion

Comparison of the two calculations provides additional confidence in the accuracy of both models, not only in dealing with the present problem but also in describing the many other transport processes that have been simulated previously by one technique or the other. The two simulation procedures are quite different. The stationary-grid model [9] and its antecedent [11] describe the flow of monomer and aggregate separately and explicitly recalculate the chemical equilibrium after each step of the transport simulation. For each species, the flows due to diffusion and driven transport are calculated simultaneously by a single combined expression. The distorted-grid model [8] uses separate routines for diffusion and driven transport and applies these expressions alternately to the concentration array. On the other hand, the flows of monomer and aggregate are described simultaneously by using appropriate average transport coefficients, and no separate reequilibration routine is necessary. It seems very unlikely that two modeling procedures that differ so greatly in structure would coincidentally produce identical errors. The close agreement of the results suggests that both models have simulated the approach to focusing equilibrium accurately. By inference, other applications of the models are probably equally reliable.

The difference in computing time between the two procedures is principally due to the fact that the stationary-grid model requires for precision and stability a more finely divided concentration array and shorter time intervals than does the distorted-grid procedure. The stationary-grid model simulates driven transport – electrophoresis in the present case – by moving solute across the boundaries between adjacent segments. The calculated flow from one segment into the next is proportional to the concentration of solute at the segment boundary which is found by interpolation, using a truncated Taylor's series defined by the mean concentrations in a few segments close to the boundary [11]. The segment boundaries must be closely spaced to minimize truncation errors in the interpolated concentrations which would otherwise produce serious and cumulative errors in the calculated flows. This is necessary even after compensating for "truncation diffusion" [9,15]. Close spacing of the boundaries in turn requires the choice of short time intervals for the successive steps of the simulation [11,16,17]. The distorted-grid model also includes an interpolation procedure to find the concentration to be used in computing the migration velocity of the solute at each segment boundary. In general, however, errors in the migration velocity are much less than proportional to errors in the solute concentration, and so the model can tolerate a degree of imprecision in the interpolation that would be unacceptable in the stationary-grid procedure. Since the interpolation need not be so precise, a coarser array of boundaries may be used; fewer segments are carried through the calculation, and the transport process can be broken up into a smaller number of longer time intervals.

A few changes could be made in the stationary-grid model used in this work that might reduce running time somewhat. For example, the reequilibration calculations could be done by a table-search procedure similar to the one used in the distorted-grid programs, or the program could be instructed to ignore segments that contain very small amounts of solute. It does not seem likely, however, that modifications in detail would eliminate the fundamental advantage in computing time of the distorted-grid approach.

Although the distorted-grid simulation uses computing time much more efficiently for simple aggregating solutes in rapid chemical equilibrium, the stationary-grid model is applicable to a much broader range of problems. In the form used here, the distorted-grid procedure is not suitable for describing systems that contain more than one independent component. Thus, it does not deal readily with chemically reacting systems involving unlike subunits, with self-associations mediated by ligands of low molecular weight, or with interacting solutes that relax slowly toward chemical equilibrium. All of these important cases are accessible by the stationary-grid approach [4]. Recent work suggests that the distorted-grid procedure can be modified to deal with at least some of these problems [18]. It appears likely, however, that the necessary modifications considerably reduce the advantage of the distorted-grid technique in computing efficiency, and it remains to be seen whether distorted-grid procedures can describe multi-component systems with acceptable accuracy. Examination of these questions is continuing.

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