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KINETIC CONTROL OF THREE-SPECIES ASSOCIATION REACTIONS IN GEL CHROMATOGRAPHY

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The gel chromatographic patterns of a monomer–dimer–tetramer system under kinetic control have been studied by computer simulation. In no case do the derivative curves of the concentration profiles exhibit more than bimodality in these systems and in some cases are found to exist as a single, almost symmetric, peak. The monomer–dimer reaction affects the dimer–tetramer reaction only slightly and the same is true for the effect of the dimer–tetramer reaction on that of the monomer–dimer. All systems can be fit, to a first approximation, by an empirical formula which suggests any reaction with a first order half life within one and one half or two orders of the transport time will be under kinetic control.

1. Introduction

Transport methods such as ultracentrifugation, countercurrent distribution, electrophoresis and gel chromatography have long been used by the biochemist to achieve either physical separation or to analyze one or more of the physical parameters of the biomolecule under study. Exact analytical solutions of the flow equation have not been solved for interacting systems, but many studies have been published using approximate solutions or using computer techniques to approximate their solution [1–19]. All these simulations make the approximation that equilibrium between the interacting species is obtained instantaneously. Early papers dealing with kinetically controlled systems [20,21] assume no dispersion (diffusion) and deal with either isomerizing systems or dimerizations. Recent work by Halvorson and Ackers [22] who have worked out the theory for kinetically controlled isomerizations on small zone gel chromatography and by Cann and Oates [23], Cann and Kegeles [24] and Zimmerman [25] has begun to expand upon this base.

A previous paper from this laboratory dealt with the self associating properties of a monomer in a kinetically controlled reversible equilibrium with its n-mer, the system being perturbed from equilibrium by passage through a gel chromatographic column. The present paper extends these simulations to include a three species system with transport behavior controlled by rapid equilibria, absence of interactions or kinetically controlled processes, in any combination for the sequential reaction steps.

2. Methods

The computer program is that described by Zimmerman [25] and derived from that of Cox [6–9]. The present program simulates the interaction between three species as shown in the following equation,

$$n\mathrm{P} \rightleftharpoons j\mathrm{P}_i \rightleftharpoons \mathrm{P}_n, \tag{1}$$

where P is the monomeric form, n is the number of subunits present in the n-mer and the product of the integers i and j is equal to n. All of the simulations in the present paper are for a monomer–dimer–tetramer system.

$$4\mathrm{P} \underset{k_{b_{21}}}{\overset{k_{f_{12}}}{\rightleftharpoons}} 2\mathrm{P}_2 \underset{k_{b_{42}}}{\overset{k_{f_{24}}}{\rightleftharpoons}} \mathrm{P}_4. \tag{2}$$

In all cases the system is initially at equilibrium and the ratios of $k_{f_{12}}/k_{b_{21}}$ and $k_{f_{24}}/k_{b_{42}}$ remain at their equilibrium values.

The flow equation for the gel chromatography of heterogeneous systems is [22,26]

$$J' = FC_t'/\bar{\xi} - \bar{L}\, dC_t'/dx + \text{rate terms}, \quad (3)$$

where J' is the flux, F is the flow rate, $\bar{\xi}$ is the weight average fraction of the column volume available to solute, $\bar{L}$ is the gradient average dispersion coefficient [16], x is the distance parameter, and C_t' is the total column concentration as defined by $C_t' = C_t\bar{\xi}$ [16,27] where C_t is the bulk solution value in mg/ml. It is the quantity C_t' that is monitored in scanning gel chromatography [27]. The quantity $\bar{\xi}$ is related to the size of the macromolecule through the weight average partition coefficient, σ_w: $\bar{\xi}_w = \alpha + \beta\bar{\sigma}_w$, where α and β are fractions of void volume and internal volume respectively [26].

Table 1 gives the physical parameters used in this paper. They are based on values for a 17 kilodalton monomer in reversible association with both its dimer and its tetramer while undergoing transport on a column of Sephadex G-200R at a flow rate of 1.2 ml/hr initially at a concentration of 0.1 mg/ml. The exact method of calculation of each of the parameters of table 1 is given in Zimmerman et al. [19].

The computer program KINETIC approximates the solution to the flow equation by alternating rounds of translation, dispersion, and chemical reactions. In the program these three steps are nested in DO loops with the chemical reaction step being innermost and the translation loop being outermost.

The simulation initially loads a column of unit cross-sectional area with solute creating a step function at $x = 0$, mathematically divides the column into boxes, translates the contents of each box as a unit, allows dispersion to occur between boxes and calculates the change in concentration of each species within each box with time as a function of the chemical reaction kinetics. The results are expressed as the concentration profile of the trailing boundary. Since not all of the volume of each box is available for reaction but mass must still be conserved, the kinetic equations must reflect this difference. These equations are*

$$dC_1'/dt = k_{b_{21}}C_2 - k_{f_{12}}C_1^2, \quad (4a)$$

$$dC_4'/dt = k_{f_{24}}C_2^2 - k_{b_{42}}C_4, \quad (4b)$$

$$dC_1'/dt + dC_4'/dt + dC_2'/dt = 0. \quad (4c)$$

Table 1
System parameters for gel chromatography simulations

State of aggregation	Partition coefficient, σ	Dispersion coefficient L (cm^2/min)	Radius (nm)
Monomer	0.714	3.61×10^{-4}	1.89
Dimer	0.602	3.92×10^{-4}	2.38
Tetramer	0.474	4.86×10^{-4}	3.00

3. Results and discussion

Whenever one investigates a system that has three species interacting through two equilibria, one is faced with trying to examine or determine the effect of each equilibrium individually. One method of isolating these effects is computer simulation, holding one set of parameters constant while varying the other set of parameters. In this way one can compare relative effects of each set of parameters.

As seen in figs. 1–4 the presence of three species is definitely not by itself sufficient to produce three peaks. In fact, in the case under study, when the dimer is in abundance one observes only one peak (figs. 1,2) and at first glance the peak could even be thought of as symmetrical. Even under conditions where the tetramer is present in relatively large amounts (figs. 3,4) one may still observe only one peak if the interaction between all forms is significant.

One can see that if one pair of parameters is held constant and the other pair varied over a fixed interval, the family of curves obtained all appear to be quite similar without regard to the values of the pair held constant. This can be seen by comparing curves A through C in any of the figs. 1–4, fig. 4 being an exception to be discussed later. Upon close examination, differences are present in peak heights, peak positions, peak to peak ratios, and the width of the curves.

In cases where a single peak is observed, the peak position shifts to the right as the values of $k_{f_{12}}$ and $k_{b_{21}}$ are lowered and shifts to the left if the values of $k_{f_{24}}$ and $k_{b_{42}}$ are lowered. These shifts are in the expected direction since as the interactions lessen the curve will attempt to become trimodal but with two

* Eq. (6) in the paper by Zimmerman [25] is incorrect and should read

$$dC_1/dt = (k_b C_n - k_f C_1^n)/\xi_1.$$

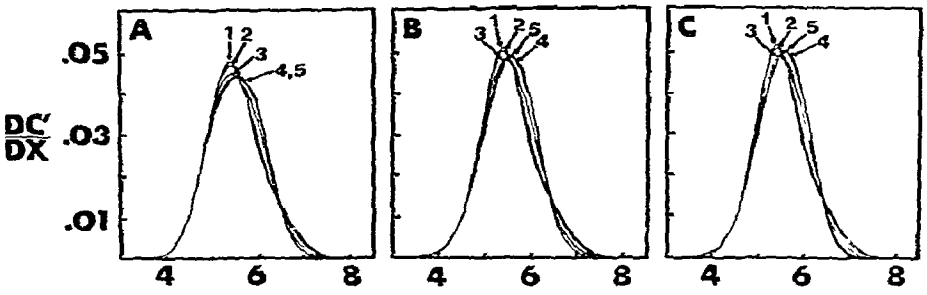

Fig. 1. 25% Monomer–50% dimer–25% tetramer. Ratios of $k_{f_{12}}/k_{b_{21}}$ = 80 ml/mg and $k_{f_{24}}/k_{b_{42}}$ = 10 ml/mg are constant in each figure. Curve A is for a constant value of $k_{b_{21}}$ = 0 min^{-1}, curve B for $k_{b_{21}}$ = 0.008 min^{-1}, and curve C is for instant equilibrium between monomer and dimer. Within each figure the $k_{b_{42}}$ values are: (1) $k_{b_{42}}$ = 0 min^{-1}, (2) 0.001 min^{-1}, (3) 0.008 min^{-1}, (4) 0.064 min^{-1}, and (5) instant equilibrium for dimer and tetramer.

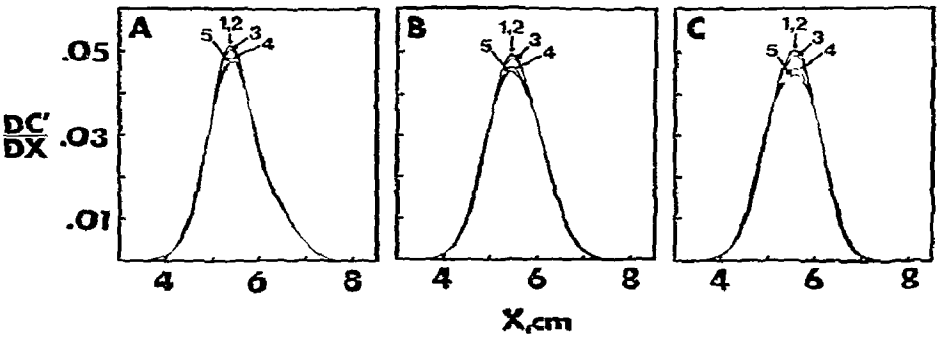

Fig. 2. 25% Monomer–50% dimer–25% tetramer. The same $k_{f_{12}}/k_{b_{21}}$ and $k_{f_{24}}/k_{b_{42}}$ ratios as in fig. 1. Curve A has a constant value of $k_{b_{42}}$ = 0 min^{-1}, curve B for $k_{b_{42}}$ = 0.008 min^{-1}, and curve C for instantaneous equilibrium between dimer and tetramer. The $k_{b_{21}}$ values are: (1) instant equilibrium between monomer and dimer, (2) 0.064 min^{-1}, (3) 0.008 min^{-1}, (4) 0.001 min^{-1}, and (5) 0 min^{-1}.

of the maxima existing only as shoulders they become obscured.

In the cases where two peaks occur, or one sees one peak with a prominent shoulder, the same type of phenomenon is observed, each peak or shoulder not only shifts relative heights but also peak positions. If the patterns are superimposed one can see a slight shift to the left in the tie point as $k_{b_{42}}$ and $k_{f_{24}}$ decrease with a corresponding shift to the right in the peak positions.

In a previous paper Zimmerman [25] defined a variable Z as

$$Z = \frac{(dC'/dx)_x - (dC'/dx)_{x_{NI}}}{(dC'/dx)_{x_{eq}} - (dC'/dx)_{x_{NI}}}, \tag{5}$$

where $(dC'/dx)_x$ is the value of the derivative curve at some reference point x, $(dC'/dx)_{x_{NI}}$ is the value of the derivative curve for the noninteraction case at reference point x, and $(dC'/dx)_{x_{eq}}$ is the value of the derivative curve for the instant equilibrium case at the reference point x. This parameter has proven to be a useful empirical relationship between the observed derivative curves and the rate equation for a monomer–n-mer equilibrium.

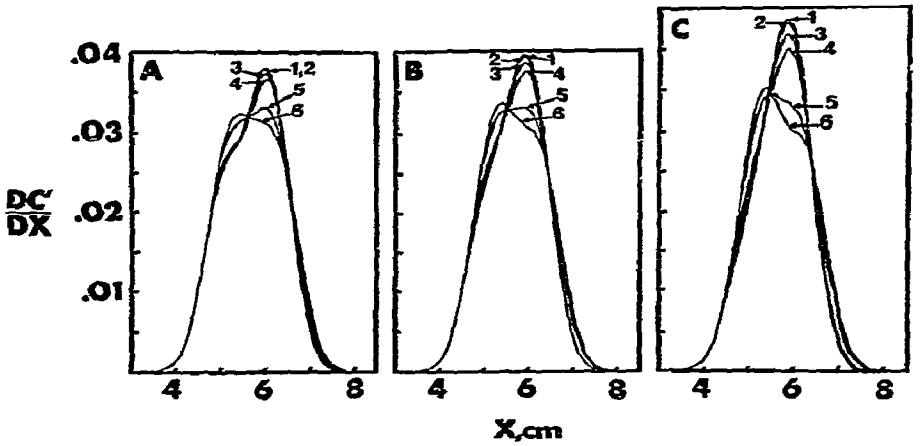

Fig. 3. 25% Monomer–25% dimer–50% tetramer. Ratios of $k_{f_{12}}/k_{b_{21}}$ = 40 ml/mg and $k_{f_{24}}/k_{b_{42}}$ = 80 ml/mg are constant in each figure. Curve A is for $k_{b_{21}}$ = 0 min^{-1}, curve B for $k_{b_{21}}$ = 0.004 min^{-1}, and curve C for instant equilibrium between monomer and dimer. The $k_{b_{42}}$ values are (1) instant equilibrium between dimer and tetramer, (2) 0.064 min^{-1}, (3) 0.016 min^{-1}, (4) 0.008 min^{-1}, (5) 0.001 min^{-1}, and (6) 0 min^{-1}.

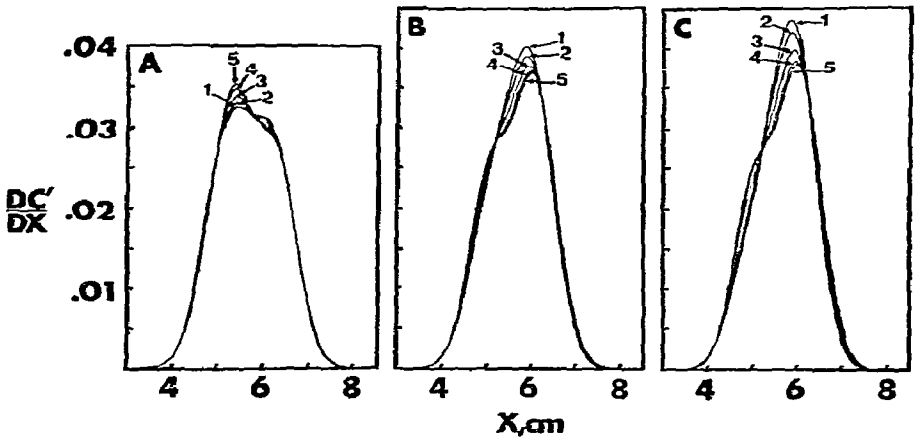

Fig. 4. 25% Monomer–25% monomer–50% tetramer. The same $k_{f_{12}}/k_{b_{21}}$ and $k_{f_{24}}/k_{b_{42}}$ values as in fig. 3. Curve A is for a constant value of $k_{b_{42}}$ = 0 min^{-1}, curve B for $k_{b_{42}}$ = 0.008 min^{-1}, and curve C for instant equilibrium between dimer and tetramer. The $k_{b_{21}}$ values are: (1) instant equilibrium between monomer and dimer, (2) 0.016 min^{-1}, (3) 0.004 min^{-1}, (4) 0.001 min^{-1}, and (5) 0 min^{-1}.

A minor modification of this relationship given below also fits the case under study in this paper.

$$G = k_b[(1-Z)/Z]^n, \tag{6}$$

where G is an arbitrary constant, k_b is the backward rate constant of the reaction under study, and n is a coefficient less than or equal to one. This equation can best be analyzed when put in a linear form

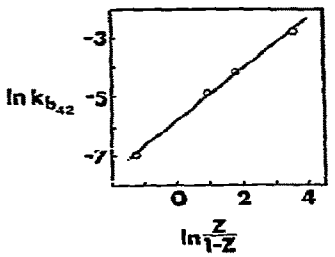

Fig. 5. Plot of data in fig. 3A according to eq. (7). This is a system initially 25% monomer–25% dimer–50% tetramer maintaining a rapid equilibrium between monomer and dimer and varying the values of the rate constants between the dimer and tetramer.

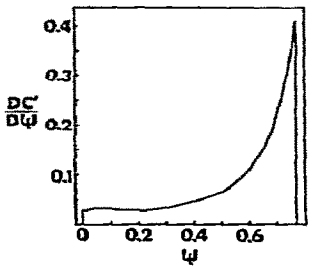

Fig. 6. Asymptotic solution for 25% monomer–25% dimer–50% tetramer. The physical constants are the same as in curve C1 for fig. 3.

$$\ln k_b = \ln G + n \ln [Z/(1-Z)] \qquad (7)$$

allowing determination of G and n. The curve picked to illustrate this analysis (fig. 5) is one of the more nonlinear curves obtained. It does illustrate, however, a common feature of all of the curves. The empirical relationship of eq. (6) is not adequate. The data in all cases shows a systematic deviation from linearity.

The values obtained for G were not constant as had been previously suggested [25] but varied from values of 6.43×10^{-3} min^{-1} to 2.54×10^{-3} min^{-1} which correspond to half lives of 107 min and 287 min respectively. Each slope was less than one (0.73 to 0.98) and had a correlation coefficient of 0.99 or higher. The value of L obtained showed definite trends with any one data set represented by any of the figs. 1–4 but the trends from figure to figure were not totally consistent. The data has been examined using relaxation mathematics but no adequate fit has yet been made.

Fig. 6 shows the asymptotic solution for the monomer–dimer–tetramer system with $K_1 = 40$ ml/mg and $K_2 = 80$ ml/mg (25% monomer–25% dimer–50% tetramer at a total protein concentration of 0.1 mg/ml – the protein system is the same as in curve C1 of fig. 3). The abscissa is written in terms of a time independent coordinate, ψ, defined as

$$\psi = (x - X_1)/(X_4 - X_1),$$

where X_1 and X_4 are the positions of the centroid of the monomer and tetramer respectively at the time of measurement. This curve is quite similar to that of Cox [9] for a rapidly equilibrating monomer–dimer–tetramer system in the ultracentrifuge. This similarity even extends to the bump seen at low ψ.

Halvorson and Ackers [22] found that in an isomerizing small zone system, the dispersion arising from chemical reaction is quite significant. We have done simulations which excluded dispersion to test this point. We find that the dispersion due to chemical reaction to be quite significant but still not the major factor in determining the contribution of dispersion to the shape of the reaction profile.

Although the data given in this paper does not confirm the simple relationship between the time the macromolecule is on the column and the rate constants involved in the reaction as had been suggested [25], the general conclusion is still valid and of importance to experimentalists as well as those who do computer simulations on transport systems. When the half life of the dissociation is within one and a half to two orders of magnitude of the transport time, the observable profiles will be under kinetic control.

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