BASIC programming are accommodated. A first-order rate constant error estimates are typically computed in <5 s for 101 data points.

RESULTS

The kinetics of iodoacetamide-blocked bovine serum albumin (IA-BSA) refolding have recently been characterized by stopped-flow monitoring of the protein's tryptophan fluorescence after a rapid pH jump (1). We reexamined this system using our microcomputer-interfaced stopped-flow instrument.

Fig. 1 shows computer-acquired and -plotted data taken with good signal-to-noise ratio (S/N) (1A), poor S/N (1B), and with computer-controlled ensemble averaging of the noisy data (1C). The computed constants are in excellent agreement with the previously reported value of 32 s^{-1} (1).

We also examined the reactions of 0.02 M $NaHCO_3$ with 0.01 N HCl (3.4×10^{-5} M bromophenol blue indicator) and of 10^{-3} M $Fe(NO_3)_3$ in 0.1 M H_2SO_4 with 1.0 M KSCN. The $NaHCO_3$ reaction yielded a rate constant of 21.1 s^{-1} (24°C), which compares well with the literature value of $19.0 \pm 2.0\ s^{-1}$ (24.1°C) (2).

For the Fe^{3+} system, four replicate runs (24°C) yielded a lifetime of 57.5 ± 0.5 ms, which compares well with an estimated value of 45 ms calculated for a slightly different ionic strength (3). The total time to acquire all four transients, display and verify their quality on the oscilloscope, compute the rate constants, and plot the observed and calculated best-fit curves on an *x-y* recorder was under 20 min.

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ON THE INTEGRATION OF COUPLED FIRST-ORDER RATE EQUATIONS

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Differential rate equations for many chemical reaction mechanisms are inherently linear or can be linearized through judicious choice of experimental conditions.

The author of this paper did not attend the actual Discussion. The present text was submitted and circulated to participants before the meeting.

Examples in biochemistry are: ligand binding to a macromolecule followed by a series of conformational changes under pseudo-first-order conditions; reversible denaturation of a protein or nucleic acid induced by a rapid change in pH; "single turnover" enzyme catalysis; pre-steady-state enzyme catalysis. In these cases the reaction mechanism can usually be written as a system of coupled first- or pseudo-first-order reactions involving n components related through a single mass conservation relationship. The time dependence of the component concentrations is given by

$$X_i = \bar{X}_i + \sum_{k=1}^{n-1} C_{ik} \exp(-\lambda_k t), \quad i = 1, 2 \ldots n, \tag{1}$$

where $\bar{X}_i$ denotes final equilibrium concentrations, the λ_k are macroscopic rate constants, and the C_{ik} are integration constants which depend on initial concentrations (X_i^0). Eq. 1 holds for sequential, branched, and cyclic reaction mechanisms.

The n differential rate equations are defined by the "coefficient matrix" **A** (1). It proves convenient to reduce **A** to an $(n-1) \times (n-1)$ matrix **A'** by eliminating one of the rate equations with mass conservation. The macroscopic rate constants are then the $n-1$ roots of the secular equation,

$$|\mathbf{A}' - \lambda \mathbf{I}| = 0 \tag{2}$$

The present communication describes a computational method that considerably simplifies the derivation of the analytical expressions of Eq. 1. The procedure involves: construction of the transpose of the $n \times n$ secular determinant, $|\mathbf{A}^T - \lambda \mathbf{I}|$, by examination of the kinetic model; reduction to $|\mathbf{A}' - \lambda \mathbf{I}|$ by examination of $|\mathbf{A}^T - \lambda \mathbf{I}|$; derivation of the $\bar{X}_i$ and C_{ik} by systematic evaluation of subdeterminants of $|\mathbf{A}^T - \lambda \mathbf{I}|$.

The first step in the calculations is to write the kinetic mechanism as a set of coupled first-order reactions, with the elementary steps and microscopic rate constants defined as

$$X_i \underset{k_{ji}}{\overset{k_{ij}}{\rightleftharpoons}} X_j \quad i \neq j;\ i, j = 1, 2 \ldots n. \tag{3}$$

The species to be eliminated by mass conservation is written X_n; otherwise the numbering of components is arbitrary. Pseudo-first-order reactions are denoted by primed rate constants, k'_{ij}. Steps resulting in formation of a product other than an X_i must be irreversible, or the product must be present in sufficient excess to allow the reverse reaction to be written as a pseudo-first-order process.

The determinant $|\mathbf{A}^T - \lambda \mathbf{I}|$ is constructed in essentially two steps: (*a*) the typical off-diagonal element in the ith row and jth column is written as $-k_{ij}$; (*b*) the diagonal element of row i is formed by taking the sum of all off-diagonal elements of row i, multiplying by -1, and subtracting λ. Then the reduced secular determinant is formed by: (*a*) subtracting each element in the last row of $|\mathbf{A}^T - \lambda \mathbf{I}|$ from all remaining elements of the same column; (*b*) striking out the last column and last row of the new determinant, followed by transposing to $|\mathbf{A}' - \lambda \mathbf{I}|$.

Finally, the constants $\overline{X}_i$ and C_{ik} are derived from initial concentrations and elements of determinant $|\mathbf{A}^T - \lambda\mathbf{I}|$. It can be shown that[1]

$$\overline{X}_i = B_i^{(0)} \Big/ \prod_l \lambda_l; \quad C_{ik} = \frac{B_i^{(k)}}{\lambda_k \prod_{l \neq k} (\lambda_k - \lambda_l)}, \tag{4}$$

where the index l runs from 1 to $n - 1$. The coefficients $B_i^{(0)}$, $B_i^{(l)}$, ... $B_i^{(n-1)}$ are explicit functions of specific rate constants, initial concentrations and the roots of Eq. 2. The superscript ($k = 0, 1, 2 \ldots n - 1$) indicates that $B_i^{(k)}$ is evaluated using λ_k. By definition we set $\lambda_0 = 0$. Conveniently, the $B_i^{(k)}$ for a given component X_i are formally identical, differing only in the definition of λ_k:

$$B_i^{(k)} = \sum_{j=1}^{n} (-1)^{1+j} \Delta_{ij}^{(k)} x_j^0, \tag{5}$$

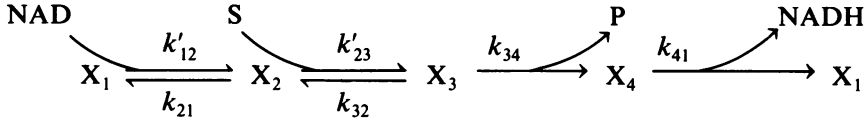
where $\Delta_{ij}^{(k)}$ is the subdeterminant obtained by striking out the ith row and jth column of $|\mathbf{A}^T - \lambda_k\mathbf{I}|$. Often, only one X_j^0 will have a finite value at the moment the reaction is initiated. The computational labor in deriving expressions for $\overline{X}_i$ and C_{ik} is then reduced to evaluating a single subdeterminant for each component.

The time dependence of products that do not feed back into the sequence of formal first-order reactions is calculated by direct integration of the precursor concentration. If product P is formed irreversibly from component X_i, we may use the following general relation:

$$P = \frac{(k_{ij} B_i^{(0)})t}{\prod_l \lambda_l} + \sum_{k=1}^{n-1} \frac{k_{ij} B_i^{(k)} (1 - \exp(-\lambda_k t))}{\lambda_k^2 \prod_{l \neq k} (\lambda_k - \lambda_l)}. \tag{6}$$

Eq. 6 is always valid in the early stages of reaction, where the rate of $P \rightarrow X_i$ transformation can be ignored.

As an example of a practical calculation using the above method, consider the derivation of the integrated rate equation for product appearance in a two-product, two-substrate enzyme reaction, such as



where X_1 = E, X_2 = E-NAD, X_3 = E-NAD-S, and X_4 = E-NADH. From inspection of the above scheme we construct the 4 × 4 determinant $|\mathbf{A}^T - \lambda\mathbf{I}|$,

$$\begin{vmatrix} k'_{12} - \lambda & -k'_{12} & 0 & 0 \\ -k_{21} & k_{21} + k'_{23} - \lambda & -k'_{23} & 0 \\ 0 & -k_{32} & k_{32} + k_{34} - \lambda & -k_{34} \\ -k_{41} & 0 & 0 & k_{41} - \lambda \end{vmatrix}$$

[1] D. Thusius. To be published.

which is reduced by examination to $|\mathbf{A}' - \lambda\mathbf{I}|$:

$$\begin{vmatrix} k'_{12} + k_{41} - \lambda & k_{41} - k_{21} & k_{41} \\ -k'_{12} & k_{21} + k'_{23} - \lambda & -k_{32} \\ 0 & -k'_{23} & k_{32} + k_{34} - \lambda \end{vmatrix}$$

When applied to enzyme reactions, Eq. 6 gives the pre-steady-state (exponential terms) and steady-state (linear term) appearance of products. If the early phase production of NADH is required, we set $i = 4$ and derive the analytical expression for $B_4^{(k)}$ from initial concentrations and the subdeterminants $\Delta_{4j}^{(k)}$ of $|\mathbf{A}^T - \lambda\mathbf{I}|$. When the reaction is initiated by adding a mixture of substrate and NAD to enzyme, the initial conditions are defined by: $X_2^0 = X_3^0 = X_4^0 = 0$; $X_1^0 = X_{\text{total}}$. Thus, the integration coefficients for NADH production in this case are completely determined with $\Delta_{41}^{(k)}$. It is readily shown that,

$$B_4^{(k)} = -\Delta_{41}^{(k)} X_1^0 = k'_{12} k'_{23} k_{34} X_{\text{total}}. \tag{7}$$

Substitution of the above relation into Eq. 6 gives the expression for initial appearance of reduced coenzyme:

$$\frac{(\text{NADH})}{X_{\text{total}}} = k'_{12} k'_{23} k_{34} k_{41} \left[\frac{t}{\lambda_1 \lambda_2 \lambda_3} + \frac{1 - \exp(-\lambda_1 t)}{\lambda_1^2(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} \right.$$

$$\left. + \frac{1 - \exp(-\lambda_2 t)}{\lambda_2^2(\lambda_2 - \lambda_1)(\lambda_2 - \lambda^3)} + \frac{1 - \exp(-\lambda_3 t)}{\lambda_3^2(\lambda_3 - \lambda_1)(\lambda_3 - \lambda_2)} \right].$$

The macroscopic rate constants are given implicitly as the roots of the secular polynomial (Eq. 2). If the roots have very different values, one can generally factorize $|A' - \lambda I|$ to obtain the λ_k in terms of the k_{ij}.[1] At the same time, the limiting conditions $\lambda_k \gg \lambda_l$ lead to considerable simplification of the denominators in the pre-exponential terms.

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