ability to reduce the mixing dimensions as much as possible. Optical detection of the progress of the reaction is accomplished by measuring the fluorescence of the liquid jet at different positions along the emerging stream.

KINETIC TRANSIENTS

A Wedding of Empiricism and Theory

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The purpose of the study of transient-state kinetics is to separate events in time and thus contribute to a detailed understanding of underlying mechanism. We have recently developed some simple but novel methods to achieve this, including the rapid-flow calorimetric determination of the kinetics of oxime formation (1) as a model for imine formation in enzymatic reactions, the stopped-flow ultraviolet spectrophotometric measurement of hydrogen exchange in nucleotides and of the effect of binding by dehydrogenases on that process (2), and the observation of transient features of enzymatic reactions over a period of minutes by using the cryoenzymological approach of Douzou and Fink (3). Thus, either by mixing and observing rapidly or by slowing the reaction sufficiently we can observe and characterize complex behavior ordinarily inaccessible to steady-state or relaxation kinetics techniques.

Many qualitative empirical conclusions can be obtained from studies of kinetic transients: "lags" give information about the kinetics of formation of precursor complexes; the existence of a "burst" suggests that there is a slow step late in the mechanism, possibly involving the formation of tight product complexes; time-dependent spectral shifts often help identify reaction intermediates; and preincubation effects (or lack thereof) give clues about slow steps and obligatory pathways in biochemical reactions. All of these phenomena can yield detailed information about the relative rates of formation and breakdown of complexes with effectors.

Quantitative mathematical and theoretical treatment of biochemical transients and related fast reactions is required, however, to give flow methods their full power as tools for the determination of mechanism. Although detailed treatments are available and many transient kinetics studies have been performed, some powerful but simple empirical methods for obtaining and handling data to yield a quantitative understanding of the processes underlying observed transients far from equilibrium have not been fully exploited. Two such approaches are developed and illustrated here with applications to the study of the glutamate dehydrogenase-catalyzed reaction, relating empirical observation to mechanistic description.

The first method is the study of the initial velocities of transients. These may be measured either directly for initially linear time-courses or by extrapolation of nonlinear experimental time-course curves to an empirically determined experimental time

zero. Once obtained, these velocities are usually susceptible to analysis by standard kinetics methods, with the steady-state formalism of enzyme kinetics often being the most powerful. The advantage of the measurement of initial transient velocities is that one thereby can isolate the very initial reaction steps of complicated mechanisms, selectively avoiding the effects of reverse reaction steps and later complex transient behavior.

In the oxidative deamination of L-glutamate by $NADP^+$ and glutamate dehydrogenase, there is an initial transient burst of reactivity, producing reduced nicotinamide absorbance of enzyme-NADH product complexes in the 340-nm region. Lineweaver-Burk plots of initial transient velocities (4) are linear in both substrate and coenzyme concentration, obeying a concentration dependence of the form:

$$e/v = 0_0 + [0_1/(NADP^+)] + [0_2/(\text{L-Glu})] + [0_{12}/(NADP^+)(\text{L-Glu})]. \quad (1)$$

Since no preincubation effects are observed, and dissociation constants for binary enzyme-$NADP^+$ and enzyme L-glutamate complexes (which can be calculated from the 0_i values obtained by fitting Eq. 1) agree with those measured independently at equilibrium, it can be concluded that both binary complexes are formed and equilibrate rapidly with coenzyme and substrate on the stopped-flow time scale. One may also calculate the concentration of material tied up in ternary enzyme-$NADP^+$-L-glutamate complexes and an apparent heterotropic cooperativity of formation of such complexes. Using the pH dependence of the 0 values, it can be shown that a ternary complex also equilibrates rapidly, with dissociation constants equal to the limiting Michaelis constants determined experimentally (5).

Not all initial velocity experiments can be handled so simply by steady-state techniques. In some cases, especially when preincubation effects are observed, it is necessary to solve the initial velocity rate equations in a quasi-equilibrium or quasi-steady-state approximation coupled to a relatively slow transient process. Such is the case for the product inhibition by α-ketoglutarate of the burst described above. In this case, the catalytic reaction is inhibited by a tight dead-end enzyme-$NADP^+$-α-ketoglutarate complex, which forms and breaks down in times commensurate with the duration of the transient burst (6).

The second, more commonly used approach to kinetic transients is the measurement of the apparent first-order rate constants that characterize the transient phenomena. It has not been generally recognized, however, that such data, even far from equilibrium, may be treated by using the powerful formalism already developed for relaxation kinetics. This is true not only for systems approaching equilibrium but also for a first-order transient approach to a steady state. This latter application is particularly valuable to the biochemical kineticist. With this approach, it has been demonstrated quantitatively that the increase of the apparent first-order rate constant for the L-glutamate deamination burst in the presence of the product inhibitor NH_4^+ is caused by the formation of a new stable intermediate in rapid equilibrium with a product

complex of enzyme, NADH, and α-ketoglutarate, previously characterized at equilibrium (7).

Using these powerful theoretical tools, coupling the results of transient kinetics with those obtained at equilibrium, and extending them over a range of solvents and temperatures, one can construct a detailed picture of biochemical mechanism, complete with both free energy and enthalpy characterization of important reaction intermediates.

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A METHOD FOR DETERMINING THE KINETIC TYPE OF FAST KINETIC DATA

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Several well-established methods exist for the treatment of data obtained by fast kinetic techniques, whereby the appropriate kinetic parameters (e.g., rate constants) may be extracted. Typically, these methods involve an initial assumption as to the appropriate type of kinetics, which is then employed to fit the corresponding kinetic equations to the data. Particularly in the case of pulse radiolysis and flash photolysis, the frequent occurrence of mixed-order processes (whether independent or competing, growth or decay), of simultaneous detection of more than one species, and of residual base-line concentrations often make it difficult to choose the correct initial assumption for data analysis.