Furthermore, a given data set can frequently be fit with comparable success by more than one type of kinetics, making it necessary to vary the experimental conditions to distinguish among the possible analyses. The systematic application of several different data reduction techniques to several sets of data becomes tedious, and the sheer volume of results thus obtained may actually obscure rather than clarify the correct solution to the problem.

We present a data reduction technique applicable without any initial assumptions regarding the kinetics involved, which produces a graphical output from whose shape the type of kinetic analysis appropriate to the data can, in most cases, be determined by simple visual examination. This technique is intended as a preliminary to the fitting and extraction of kinetic parameters. Let $V(t)$ be the data trace proportional to concentration, and let $\mathrm{d}/\mathrm{d}t\,[\log(V(t_0)/V(t)0]$ be plotted vs. $V(t)$. The resulting transform is linear in the special case of competing first- and second-order processes with the intercept and slope giving the first- and second order-rate constants, respectively (1, 2). For more complicated kinetics this transform may be sigmoid or otherwise curved, but it is generally quite distinctive.

Computation of the transform described involves the difficult problem of evaluating the derivative of experimental data containing noise. Acceptable results were obtained by first applying a simple linear filter to the function to be differentiated. Then a least-squares fit of the first eight Chebyshev polynomials was obtained and differentiated analytically.

Results are presented for synthetic data of various types, and the limiting signal-to-noise ratio for successful analysis is examined. The method is also applied to experimental data and the usefulness of families of such curves, corresponding to different experimental conditions, is illustrated.

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THE EFFECT OF PRETREATMENT WITH CALCIUM AND MAGNESIUM IONS ON PHOSPHOENZYME FORMATION BY SARCOPLASMIC RETICULUM ATPase

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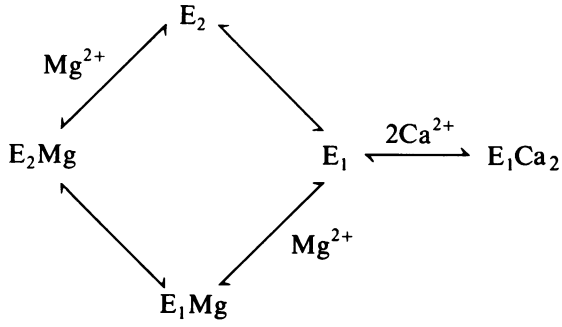
It has previously been shown (1, 2) that pretreatment of $(Na^+ + K^+)$ATPase with K^+ (10–20 mM) before the addition of Na^+ and ATP slows the rate of phosphoenzyme formation and reduces the early phosphate "burst." These effects have been explained

as resulting from the slow conversion of E_2, a form of the enzyme stabilized by high K^+ to E_1, a form stabilized by Na^+ and rapidly phosphorylated by ATP. Having Na^+ present initially with K^+ overcomes the effect resulting from pretreatment with K^+ alone by a mechanism postulated to involve Na^+-induced conversion of E_2 to E_1. To determine whether analogous effects occur in the reactions catalyzed by sarcoplasmic reticulum ATPase, the kinetics of ^{32}P incorporation were investigated after pretreatment with high levels of Mg^{2+}. Rapid mixing was carried out with a quench-flow apparatus (3), which mixes the enzyme and substrate solutions at a volume ratio of 1 to 20. This arrangement was used to bring about the dissociation of Mg^{2+} from the enzyme and to minimize possible competitive interactions between the ligands. If the enzyme is pretreated with 1 mM Mg^{2+} and 1 mM EGTA to remove tightly bound Ca^{2+}, phosphoenzyme formation resulting from the addition of 10 μM ATP and 50 μM Ca^{2+} is rapid ($t_{1/2}$ = 15 ms) and displays a transient overshoot. Pretreatment of the enzyme with 20 mM Mg^{2+} and 1 mM EGTA reduced the rate of phosphorylation ($t_{1/2}$ = 28 ms) and eliminated the overshoot without affecting the steady-state level of phosphoenzyme. A small amount of Ca^{2+} ($\sim$10 μM) included in the enzyme medium with 20 mM Mg^{2+} was able to restore completely the rapid rate of phosphorylation obtained when 1 mM Mg^{2+} is present initially. Excluding both Ca^{2+} and Mg^{2+} from the enzyme medium resulted in a high rate of phosphorylation ($t_{1/2}$ = 13 ms) and no overshoot. These findings are interpreted with the aid of the following diagram:



where, by analogy to the events described in $(Na^+ + K^+)$ATPase, the unliganded enzyme exists in two separate conformational states characterized by having either high affinity for Ca^{2+} (E_1) or low affinity for Mg^{2+} (E_2). In the absence of ligands, E_1, the form of the enzyme rapidly phosphorylated by ATP, is favored. High Mg^{2+} drives the equilibrium in favor of E_2; upon addition of Ca^{2+} and ATP, E_2 is converted to E_1 at a rate slower than the rates of substrate binding and phosphorylation.

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