Effect of Dissociation and Association on Enzyme Reaction Kinetics

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This note is concerned with the effects of enzyme inactivation, due either to dissociation into inactive subunits or association into aggregates, on the kinetics of enzyme catalyzed reactions. Reithel (1963) has pointed out, in a detailed review, that the average globular protein is a structure of molecular weight 10^5 or greater which usually consists of several structural subunits and whose biological (enzymic) activity ordinarily vanishes upon dissociation into the subunits. Likewise, enzymic activity can also disappear if aggregation (or association) into larger entities occurs; however, this is less common than dissociation.

Association and dissociation appear to be very widespread phenomena in relation to proteins. Many studies have disclosed that only moderate environmental changes are requisite for systematically and reproducibly affecting protein association and dissociation. The state of solution dilution or concentration, pH, temperature, and ionic strength are key factors. A great number of reagents and specific ions are important, particularly urea, hydrocarbons, long chain alcohols, detergents, chelating agents, steroids, certain salts, and various polyelectrolytes. The dissociation of enzymes either by dilution or by chemical agents appears to be quite reversible, in general.

While the catalytic activity of an enzyme has no direct and universal relation to the state of aggregation, much evidence of a general correlation exists. In particular, there is a great deal of evidence (Reithel, 1963) that many enzymes are special complexes of either identical subunits or of unlike subunit chains of very different configuration, and that catalytic activity depends crucially on the exact juxtaposition of the subunits.

The effect of dilution on dissociation is especially well documented. Bernfeld et al. (1954, 1965) have presented extensive data showing the variation of specific activity with enzyme concentration for β-glucuronidase, aldolase, lactic dehydrogenase, and two types of amylases. These enzymes were shown to decrease very substantially and steadily in activity upon increasing dilution. According to Bernfeld et al. (1965), this behavior "can best be explained by dissociation of the enzymes into enzymically inactive products." These authors also showed that various macromolecular polycations were capable of preventing or reversing such dissociation.

Other studies (for example, Charlwood et al., 1961) have shown more specifically how the proportions of monomers, dimers, etc., change with enzyme concentration, and have confirmed that dissociation does often accompany dilution. Reithel (1963, p. 211) has indicated that association and dissociation can be viewed as states of dynamic equilibrium in which "some partially disoriented subunits (are) in equilibrium with some intact subunits and the assembled complex." Thus, it has been found that polymeric forms ranging mainly from dimers to hexamers (but rarely larger) are often encountered.

The foregoing evidence clearly indicates that for many enzymes the association of two or more like or unlike subunits is needed for catalytic activity, and that association/dissociation is a dynamic equilibrium process which is affected in reproducible ways by a variety of factors, the most universal and simple of which is ordinary dilution.

THEORETICAL MODEL FOR EFFECT OF DISSOCIATION

The effect of dissociation on enzyme kinetics can be quantitatively modeled as follows. Assuming that an active enzyme E consists of two subunits (E_1 and E_2) which are inactive, that a dynamic equilibrium exists between E and its subunits, and that the enzyme reaction which E catalyzes is governed by Michaelis-Menten kinetics, we may write

$$E \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E_1 + E_2 \tag{1}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} P \tag{2}$$

We know that the total enzyme concentration E_o (in moles per liter) is constant; that is

$$(E_o) = (E) + (ES) + \frac{1}{2}(E_1) + \frac{1}{2}(E_2) \tag{3}$$

Since $(E_1)(E_2)/(E) = k_3/k_{-3} = K_3$, and by noting that (E_1) and (E_2) must be equal, it is clear that $(E_1) = (E_2) = [K_3(E)]^{1/2}$. But, from classical Michaelis-Menten theory we know that $(ES)/[(E)(S)] = k_1/(k_{-1} + k_2) = 1/K_m$, and therefore $(E) = K_m(ES)/(S)$. The right side of Equation (3) can therefore be written in terms of only (ES) through use of these relationships. One may thus obtain

$$(E_o) = [1 + K_m/(S)](ES) + [K_3K_m/(S)]^{1/2}(ES)^{1/2} \tag{4}$$

Note here that in the absence of enzyme dissociation ($K_3 = 0$), the derivation would lead to an expression for simple Michaelis-Menten kinetics. Solving this quadratic equation and rearranging the result into a convenient form allows one to express the reaction rate v as

$$v = -d(S)/dt = k_2(ES)$$

$$= \frac{k_2K_3K_m(S)}{4[K_m + (S)]^2}\left[\sqrt{1 + \frac{4E_o[K_m + (S)]}{K_3K_m}} - 1\right]^2 \tag{5}$$

The reaction rate relative to the rate which would pertain in the absence of dissociation ($K_3 = 0$) can be obtained by dividing the above result by $k_2E_o(S)/[K_m + (S)]$, the classical Michaelis-Menten reaction rate expression. One thereby obtains

$$\phi = \frac{\text{rate with dissociation}}{\text{rate without dissociation}} = [(\theta + 1)^{1/2} - \theta^{1/2}]^2 \tag{6}$$

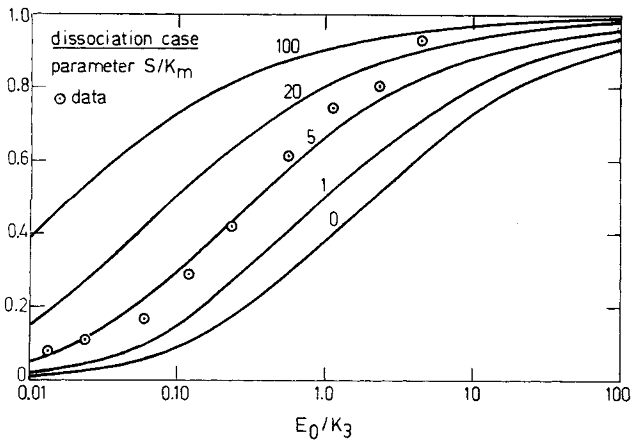

Fig. 1. Effect of enzyme dissociation on relative reaction rate.

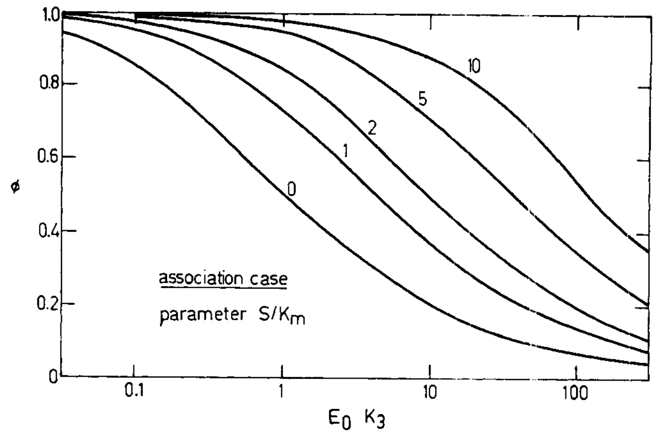

Fig. 2. Effect of enzyme association on relative reaction rate.

where $\theta = K_3K_m/4E_o[K_m + (S)] = (K_3/4E_o)/[1 + (S)/K_m]$. Clearly, this result suggests that one can plot ϕ as a function of the dimensionless enzyme concentration E_o/K_3, with $(S)/K_m$ as a parameter. Figure 1 is such a plot. One can deduce that the reduction in reaction rate due to dissociation will generally be significant only if the degree of dissociation is large, or if (S) or E_o is small. For example, if one initially has an E_o mole per liter enzyme solution, and the enzyme dissociates by 27%, then $K_3 = (0.27E_o)(0.27E_o)/0.73E_o = 0.100E_o$ and $K_3/E_o = 0.100$ (note that the particular magnitude of E_o does not matter). If $(S)/K_m = 1$, a reasonable value, the loss of activity, as seen from Figure 1, will be only about 20%. It might be noted finally that, for $K_3 = 0$, the case of zero dissociation, $\theta = 0$, and from Equation (6) the value of ϕ is 1, as it should be.

COMPARISON WITH EXPERIMENTAL DATA

The experimental data of Bernfeld et al. (1954) on the specific activity of calf spleen β-glucuronidase, in micromoles of substrate (biphenylglucuronic acid) split per mg of enzyme per hour, versus enzyme concentration in micrograms per ml permit an approximate test of the above theory for dissociation to be made. Since $\phi = v[K_m + (S)]/k_2E_o(S)$, and the term "specific activity" is identical to v/E_o, then $\phi = (\text{specific activity})/[k_2(S)/(K_m + (S))]$. The data of Bernfeld et al. (1954) show clearly that for a very low degree of dissociation ($\phi \rightarrow 1$) a specific activity value of ~ 210 is obtained. Hence, we can assume that $k_2(S)/[K_m + (S)] \cong 210$, even though we do not know k_2 and K_m individually. Using this information, the specific activity values of Bernfeld et al. have been converted to ϕ values. These are plotted in Figure 1.

Although we do not know K_3 either, we have observed that a value of $K_3 = 7.9$ micrograms per ml places their data, with respect to the abscissa scale, in the positions shown in Figure 1. Acknowledging that this value of K_3 is not independently confirmed (and the correct left-to-right position of the data therefore not proven), it nevertheless is clear that the general shape of the data trend agrees with that predicted theoretically.

ACTIVITY LOSS DUE TO ASSOCIATION

Just as catalytic activity can be lost through dissociation, likewise activity can be lost via the aggregation of proteins into larger than normal *in vivo* complexes. Reithel (1963) has pointed out, for example, that proteins containing many sulfhydryl groups can easily form disulfide cross-linked polymers when subjected to an oxidative environment. Such aggregation generally leads, of course, to inactivation.

A major way in which aggregation can be induced is via the use of much higher than normal enzyme concentrations. With the increasing use of enzymes in industrial-scale processes, the utilization of concentrated solutions may become more common. Depressed enzyme activity should be expected in such cases.

The effect of activity loss via aggregation can be analyzed along the same lines as was the effect of dissociation. Starting with the equation

$$E + E \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E_2(\text{inactive}) \qquad (7)$$

it is clear that $(E_2)/(E)^2 = k_3/k_{-3} = K_3$, and therefore $(E_2) = K_3(E)^2$. Hence

$$\begin{aligned}(E_o) &= (E) + (ES) + 2(E_2)\\ &= (E) + (ES) + 2K_3(E)^2 \qquad (8)\end{aligned}$$

Since $(E) = K_m(ES)/(S)$ from classical Michaelis-Menten theory, as noted previously, then Equation (8) can be written as

$$(E_o) = \frac{K_m}{(S)}(ES) + (ES) + \frac{2K_3K_m^2(ES)^2}{(S)^2} \qquad (9)$$

Solving this quadratic for (ES) and following the strategy of the previous development exactly, we get

$$\phi = \frac{1}{\theta}[-1 + \sqrt{1 + 2\theta}] \qquad (10)$$

where ϕ is the ratio of the reaction rate with association effects to the reaction rate without association, and $\theta = 4K_3E_o/[1 + (S)/K_m]^2$. For $K_3 = \infty$ (complete association), $\theta = \infty$, and from Equation (10) clearly $\phi = 0$ as it should. For $K_3 = 0$ (zero association), $\theta = 0$, and application of L'Hospitals rule shows that $\phi \rightarrow 1/(1 + 2\theta)^{1/2} \rightarrow 1$ as $\theta \rightarrow 0$, again as expected. ϕ can be plotted as a function of E_oK_3 with $(S)/K_m$ as a parameter, as shown in Figure 2. Note that if E_o is large, the relative reaction rate can be rather strongly diminished; however, the absolute rate would still be substantial. Unfortunately, specific data which would permit a direct test of the theory developed here for the case of enzyme association are lacking. Hence, the validity of Equation (10) is unproven. Nevertheless, since the basic concepts underlying the theoretical development are certainly sound in general, and since the previous comparison of the dissociation case theory with Bernfeld's data suggested strong support for that situation, we may at least have some confidence in the applicability of Equation (10) to real systems.

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Effects of Surface Tension and Gravity Forces in Determining The Stability of Isothermal Fiber Spinning

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Under certain conditions during the melt spinning of synthetic fibers, an instability known as draw resonance can occur (Miller, 1963). The instability can be seen as a periodic oscillation in filament thickness at a fixed point in the drawdown zone. Such a thickness oscillation increases in amplitude as the take-up speed is increased. The onset of resonance occurs at a critical value of the draw ratio (ratio of take-up speed to speed at the die exit) E_{crit}.

E_{crit}, in turn, can depend upon rheological properties of the fluid (Pearson and Shah, 1974; Fisher and Denn, 1976; Weinberger et al., 1976) and upon spinning conditions (Shah and Pearson, 1972). For example, E_{crit} for a Newtonian fluid is predicted theoretically to depend upon inertia, gravity, and surface tension forces, as well as upon thread-line cooling. However, for a Newtonian fluid spun isothermally under conditions where viscous forces dominate, the critical draw ratio E_{crit} is predicted to be a constant, 20.21 (Kase, 1966; Pearson and Matovich, 1969). This value has been recently corroborated by experimental tests with a Newtonian silicone oil (Donnelly and Weinberger, 1975).

Shah and Pearson (1972), using a linearized perturbation analysis, obtained quantitative predictions of the effects of inertia, surface tension, and gravity forces upon E_{crit}. Shah and Pearson, hereafter S-P, obtained numerical solutions to the steady state and perturbation equations of motion and continuity for the boundary conditions of constant take-up and die speeds. These solutions enabled them to generate the three functions $E_{\text{crit}}(Re)$, $E_{\text{crit}}(Re/Fr)$, $E_{\text{crit}}(Re/We)$, corresponding to the three separate cases of nonzero Re, Re/Fr, or Re/We. Here, the dimensionless groups representing the ratios of inertial, gravity, and surface tension forces to viscous forces, respectively, are

$$\begin{aligned} Re &= \frac{\rho L \bar{v}_o}{3\eta} \\ \frac{Re}{Fr} &= \frac{\rho g L^2}{3\eta v_o} \\ \frac{Re}{We} &= \frac{\sigma L}{6\eta a_o \bar{v}_o} \end{aligned} \qquad (1)$$

S-P found that E_{crit} increased with increasing Re and Re/Fr and decreased with increasing Re/We. In the limit of large Re/Fr, one approaches the conditions of a freely falling stream, and the increased stability for this case is perhaps expected. Similarly, large Re/We corresponds to large (relatively) surface tension forces, and the decreased stability for this case is likewise perhaps expected. These predictions are important since they suggest means whereby draw resonance can be avoided by suitable modification of the spinning conditions. Accordingly, it becomes important to test these predictions; the present work describes the results of such tests of the effects of gravity and surface tension forces upon E_{crit}.

EXPERIMENTAL

The experimental apparatus and procedure were identical to those used earlier by Donnelly and Weinberger (1975). Briefly, the apparatus consisted of a vertically mounted syringe pump and a variable speed takeup roll. The fluid used was a Newtonian silicone oil, $\sigma = 22.4 \times 10^{-3}$ N/m, $\eta = 100$ N s/m^2. The take-up speed was increased until thread-line oscillation could be observed, and photographs of the thread line at moments of maximum and minimum thickness (at a fixed location 40% of the distance from the die to the take-up roll) were taken at stepwise increments of the take-up speed (or E). Plots of the resulting diameter ratio DR as a function of E were extrapolated to the line $DR = 1$; the intercept thus defines E_{crit}. Temperatures were maintained at 25 ± 1°C.

The apparatus could not be readily modified to permit investigation of inertial effects (Re); however, the effects of gravity (Re/Fr) and surface tension (Re/We) could be evaluated. Re/Fr was varied from 10 to 70, while Re/We ranged from 0.2 to 0.81. Note that neither gravity nor surface tension was varied directly but rather the ratios of gravity and surface tension forces to viscous forces.

RESULTS AND DISCUSSION

The experimental conditions and results are listed in Table 1 and the results are summarized in Figure 1, where E_{crit} is plotted vs. Re/Fr, with Re/We a parameter. In all cases, $Re < 4 \times 10^{-3}$, which is well below the Re of 3×10^{-2} where inertial effects, as predicted by Shah and Pearson, should become important. Two curves, one for