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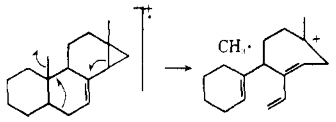
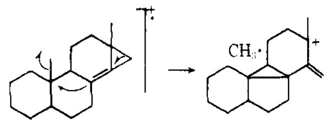


i, *m/e* 230 ii, *m/e* 215



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Cooperativity in Bimolecular Micelle-Catalyzed Reactions. Inhibition of Catalysis by High Concentrations of Detergent

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Abstract: The rate constant of micelle-catalyzed reactions as a function of detergent concentration has previously been treated as being analogous to positive cooperativity in enzymatic reactions. A kinetic model analogous to the Hill model accommodated data on the rate constants of many micellar reactions as a function of detergent concentration. Most bimolecular micelle-catalyzed reactions reach a rate maximum, which decreases with increasing detergent concentration. In an extension of the previous treatment this behavior is described in analogy with substrate inhibition of enzymatic reactions. Two terms are derived which quantitatively describe this inhibition: n' is an index of cooperativity in formation of the inactive detergent substrate complex, and $K_{n'}$ is the association constant for this complex formation. In some cases, the term n' asymptotically approaches unity at very high detergent concentration, indicating a lack of cooperativity in association under these conditions.

Reactions catalyzed by micelles have often been viewed as models of enzyme-catalyzed reactions.[1,2] This analogy is based on the gross structural similarities and properties of micelles and enzymes, and the fact that both micelles and enzymes bind substrate in a noncovalent manner prior to the catalytic step.[2] Recently, I drew an analogy between micellar reactions and reactions catalyzed by many regulatory enzymes.[3] The rate constants of micelle-catalyzed reactions when plotted vs. detergent concentration give sigmoid shaped curves. This behavior is analogous to positive cooperativity in enzymatic reactions, a sigmoid shaped dependence of velocity on substrate concentration. A kinetic model analogous to the Hill model,[4] which describes enzymatic reactions, accommodated published data on the rate constants of many micellar reactions as a function of detergent concentration.[3] This treatment assumed that a rate plateau is achieved at high detergent concentrations. While this assumption is generally valid for unimolecular spontaneous reactions, most bimolecular reactions which are catalyzed by micelles have rate maxima. At very high detergent concentration, the rates of reaction decrease with increasing detergent concentration. In an extension of the previous treatment, this behavior is now described in analogy with substrate inhibition of enzymatic reactions.

Theory and Methods

The simple mathematical model used previously[3] to describe the sigmoid shaped dependence of rate constant on detergent concentration begins with the assumption that a substrate, S, and a number, n, of detergent molecules, D, aggregate to form catalytic micelles, D_nS, which may then react to yield product:

$$\begin{gathered} n\mathrm{D} + \mathrm{S} \underset{K_\mathrm{D}}{\rightleftharpoons} \mathrm{D}_n\mathrm{S} \xrightarrow{k_\mathrm{m}} \text{product} \\ \mathrm{S} \xrightarrow{k_0} \text{product} \end{gathered} \tag{1}$$

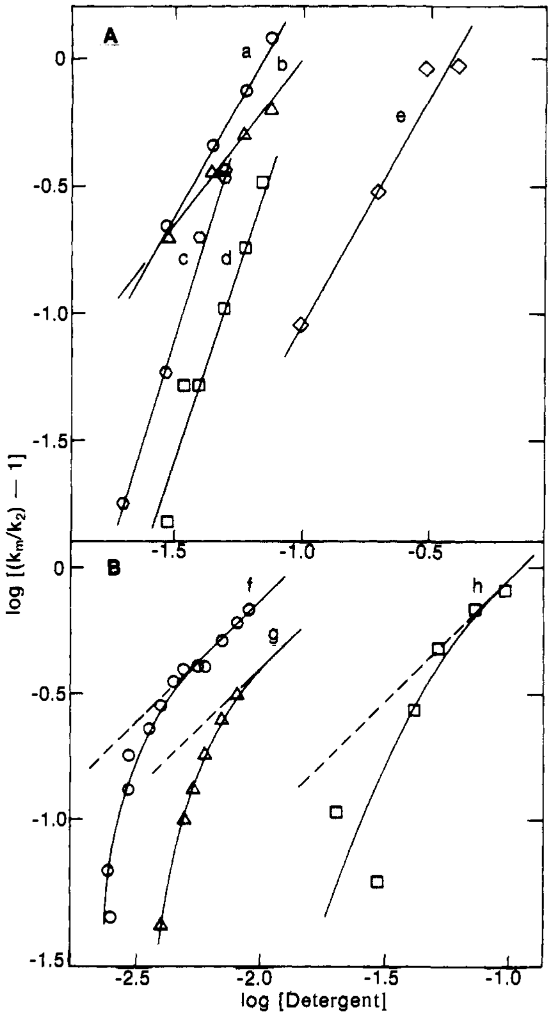

Figure 1. Plots of log $[(k_m/k_2) - 1]$ vs. log of detergent concentration according to eq 6 for bimolecular reactions catalyzed by micelles: (a) hydrogen ion catalyzed hydrolysis of methyl orthobenzoate in disodium 2-sulfooctadecyl sulfate, $n' = 1.79$, $-(\log K_{n'})/n' = -1.15$ (data from ref 14); (b) hydrogen ion catalyzed hydrolysis of methyl orthobenzoate in disodium 2-sulfoethyl-α-sulfostearate, $n' = 1.18$, $-(\log K_{n'})/n' = -0.96$ (data from ref 14); (c) reaction between hydroxide ion and 2,4-dinitrochlorobenzene in cetyltrimethylamine, $n' = 2.22$, $-(\log K_{n'})/n' = -1.16$ (data from ref 11); (d) reaction of 2,4-dinitrofluorobenzene and sodium hydroxide in cetyltrimethylamine, $n' = 3.06$, $-(\log K_{n'})/n' = -0.98$ (data from ref 15); (e) hydrogen ion catalyzed hydrolysis of methyl orthobenzoate in sodium decyl sulfate, $n' = 1.87$, $-(\log K_{n'})/n' = -0.44$ (data from ref 9); (f) reaction of p-nitrophenyl diphenyl phosphate with sodium fluoride in cetyltrimethylamine (data from ref 16); (g) reaction of p-nitrophenyl diphenyl phosphate with sodium hydroxide in cetyltrimethylamine (data from ref 16); (h) hydrogen ion catalyzed hydrolysis of methyl ortho-p-chlorobenzoate (data from ref 14).

K_D is the dissociation constant of the micelle back to its free components, k_m is the rate of reaction within the micelle, and k_0 is the rate constant of reaction not catalyzed by detergent. The observed rate constant as a function of detergent concentration is

$$k_{obsd} = \frac{k_m[D]^n + k_0K_D}{K_D + [D]^n} \qquad (2)$$

The term n is derived from experimental data as the slope of a plot of log $[(k_{obsd} - k_0)/(k_m - k_{obsd})]$ vs. log [D];[3] the intercept of the log [D] axis is $(\log K_D)/n$ and is equal to that concentration of detergent at which catalysis by detergent shows one-half its maximum effect on k_{obsd}.

The rate constants of bimolecular reactions often decrease with detergent concentration at very high detergent concentrations. This phenomenon may be treated as being analogous to substrate inhibition of an enzymatic reaction with the exception that substrate and catalyst reverse their roles so that inhibition by catalyst is seen. The overall reaction scheme which describes these bimolecular micelle-catalyzed reactions is

$$\begin{tikzcd} nD + S \arrow[r, shift left] & D_nS \arrow[l, shift left, "K_D"] \arrow[r, "k_m"] \arrow[d, shift right, "+ n'D"' near start, "K_{n'}"'] & \text{product} \qquad (3) \\ & D_nD_{n'}S \arrow[u, shift right] & \end{tikzcd}$$

$$S \xrightarrow{k_0} \text{product}$$

In this scheme, n' is the additional number of detergent molecules which must associate with the catalytic micelle, D_nS, to completely inactivate it, and $K_{n'}$ is the association constant of this interaction. The second reactant would be at a steady-state concentration and react with the catalytic micelle, D_nS, in the step defined by the rate constant k_m. One can demonstrate that the second-order rate constant, k_2, for a bimolecular reaction which is catalyzed by a detergent according to the scheme of eq 3 is

$$k_2 = \frac{k_m[D]^n + k_0K_D}{K_D + [D]^n + K_{n'}[D]^n[D]^{n'}} \qquad (4)$$

At low detergent concentrations, eq 4 reduces to eq 2. At high detergent concentrations eq 4 reduces to

$$k_2 = \frac{k_m}{1 + K_{n'}[D]^{n'}} \qquad (5)$$

This may be rearranged to

$$\log [(k_m/k_2) - 1] = \log K_{n'} + n' \log [D] \qquad (6)$$

A plot of log $[(k_m/k_2) - 1]$ vs. log [D] is linear with a slope of n'; and at log $[(k_m/k_2) - 1] = 0$, log [D] $= -(\log K_{n'})/n'$. The term $-(\log K_{n'})/n'$ is that concentration of detergent at which inhibition by detergent reduces k_2 to one-half of its maximum value, k_m.

Results and Discussion

The applicability of the treatment given by eq 3 and 4 was tested with data for micelle-catalyzed reactions which are available in the literature. Data obtained at high detergent concentrations were plotted in the form of log $[(k_m/k_2) - 1]$ vs. log [D] according to eq 6 (Figure 1). Data for many of these reactions gave linear plots (Figure 1A). Values of n' which were the slopes of these lines ranged from 3.1 to 1.0. In those cases where values of n had been determined previously,[3] it was found that n' was equal to or less than n. However, there was no obvious simple correlation between n' and n. Values of $-(\log K_{n'})/n'$ were also determined. In those cases where values of $(\log K_D)/n$ had been determined previously,[3] $-(\log K_{n'})/n'$ was generally found to be 1–1.4 units greater than $(\log K_D)/n$. However, there was no simple correlation between $-(\log K_{n'})/n'$ and $(\log K_D)/n$ or between $K_{n'}$ and K_D.

Plots of log $[(k_m/k_2) - 1]$ were not always linear. Some curved noticeably and reached limiting slopes of $n' = 1.0$ at very high detergent concentrations (Figure 1B). That this change of slope is seen should not be surprising. The model of micelle formation, and inhibition of catalysis by detergent as presented in eq 3 and 4, simplifies into two steps the multiple aggregation steps which must be involved in the formation of micelles. Formally n and n' describe the stoichiometries of the aggregation reactions given by eq 3 and 4. Functionally it is probably more accurate to consider n an "index of cooperativity" in formation of catalytic micelle, D_nS, and n' an "index of cooperativity" in formation of the inactive detergent-substrate complex, $D_nD_{n'}S$, by analogy with cooperativity in enzymatic reactions.[5] Clearly, cooperativity in the later steps decreases with increasing concentration. At very high detergent concentrations, there is no cooperativity in micelle formation. Only a simple association equilibrium exists whereby single molecules of detergent (as indicated by $n' = 1$) are absorbed by fully formed micelles.

This tendency toward a simple association equilibrium at very high detergent concentration in micelle formation has a parallel in enzymatic reactions which show positive cooperativity. Plots according to the Hill model[4] have slopes which approach 1.0 at very high and very low ligand concentrations.[6] The behavior of these enzymes which show cooperativity is generally interpreted in terms of the Monod–Wyman–Changeux[7] or the Koshland–Nemethy–Filmer[8] models which define extreme conformational forms of the catalyst. Slopes of 1.0 at high or low ligand concentration reflect simple noncooperative associations to the unliganded or almost completely liganded conformational forms.

To test the usefulness of the treatment given by eq 3 and 4, the derived constants n, n', k_D, and $K_{n'}$ were used to fit experimentally obtained data for the acid-catalyzed hydrolysis of methyl orthobenzoate in sodium decyl sulfate[9] (Figure 2). Equation 2 with derived values of n and K_D fit the data well at low detergent concentration, but not at higher concentration. However, eq 4 with derived values of n, n', K_D, and $K_{n'}$ fit the data at all detergent concentrations.

Two possible molecular mechanisms for inhibition of bimolecular reactions at high detergent concentration have been put forward. The first[1,10] states that once there is sufficient ionic detergent to take up all the substrate into micelles, addition of more detergent merely increases the counterion concentration, and these counterions inhibit approach of ionic reagents. The second[10-13] holds that when there is sufficient detergent for all the substrate to be taken up into the micelle, the addition of more detergent leads to deactivation of the ionic reactant. That is, ionic reactant (e.g., H^+ of OH^-) will be attracted to micelle surface areas which contain no substrate with a consequent diminution of it at the surface areas which contain substrate. The model of detergent inhibition presented in eq 3 and 4 are consistent with both of these explanations. Using the second hypothesis, Bunton and Robinson[11] devised an empirical equation to define the rate constant of bimolecular micelle-catalyzed reactions. Their treatment introduced a squared term in detergent concentration and yielded an equation similar in form to eq 4. A similar equation with an exponential term in the denominator has been put forward by Berezin et al.[13] to explain inhibition of bimolecular micellar reactions at high detergent concentration.

The model of micellar catalysis put forward by this and preceding papers[3] may be used to fit a wide variety of experimental data already in the literature. It requires a minimum number of assumptions, and does not require use of the critical micelle concentration. When applied to micellar catalysis of bimolecular reactions, it utilizes the same assumption to explain inhibition at high detergent concentration and catalysis at low detergent concentration. Both catalysis and inhibition reflect aggregation of substrate and detergent molecules.

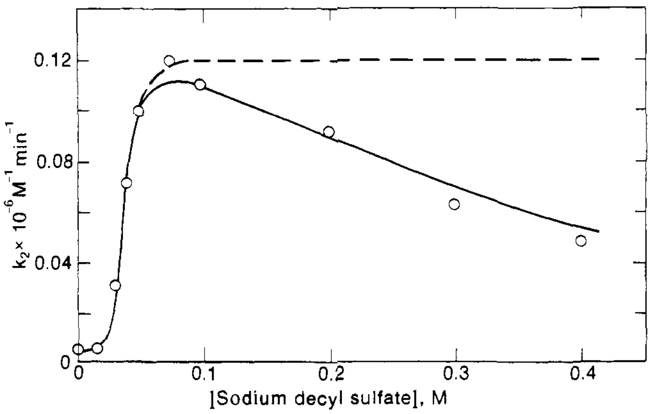

Figure 2. The hydrogen ion catalyzed hydrolysis of methyl orthobenzoate in sodium decyl sulfate. Broken line was calculated from eq 2 with $n = 5.46$ and $K_D = 1.67 \times 10^{-8}$; solid line was calculated from eq 5 with $n = 5.46$, $K_D = 1.67 \times 10^{-8}$, $n' = 1.87$, and $K_{n'} = 6.76$ (data from ref 9).

Acknowledgment. I wish to thank John Moe for his helpful criticisms and suggestions.

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