Table I. Summary of Proxyl and Doxyl Nitroxide ESR Spectra in Dodecane–Water

Nitroxide	A_0 water (g)	A_0 dodecane (g)	ΔA_0 (g)	K^a
 (proxyl)	16.37	14.02	2.35	7.27
 (doxyl)	16.02	14.32	1.70	3.32

$^a K$ = [nitroxide]$_{dodecane}$/[nitroxide]$_{water}$ calculated by digitalization and double integration of ESR spectra obtained from each of the two deoxygenated phases.

esulfonyl chloride–Et_3N in ether at 0° afforded the corresponding mesylate which was converted into quaternary amine salt **10**, a yellow solid, mp 88.5–89.5° (71%, based on **6**) (Found for **10**·0.5H_2O: C, 63.10; H, 11.44; N, 5.23) by heating (1 h) the mesylate in the presence of trimethylamine in THF at 110° (bomb).

In another series of experiments an egg lecithin–cholesterol–alcohol **6** mixture (molar ratio = 150:75:1) was dispersed in water. Qualitatively, the ESR spectrum of **6** in this system (A_{max} = 32 G) was the same as that where **6** was replaced by 7-doxylstearyl alcohol.[13] Most interestingly, whereas no ESR signal could be obtained with a sonicated mixture of 7-doxylstearyl alcohol–cardiolipin–cytochrome *c* (molar ratio = 1:70:20) in water (irreversible reduction, see above), with a comparable mixture containing proxyl alcohol **6** an ESR signal was readily observable. The signal intensity increased threefold when O_2 was briefly bubbled through the sample, demonstrating that while proxyl alcohol **6** does suffer some reduction in the cytochrome *c* preparation, the reaction is reversible to a large extent.

In order to assess the change in polarity resulting from substitution of the ring oxygen atom of a doxyl nitroxide by a methylene group in these new labels, both doxyl nitroxide **1** (R_1 = R_2 = Me) and proxyl nitroxide **4** (R_1 = R_2 = Me) were partitioned under identical conditions between dodecane and water. From the data shown in Table I it is apparent that a, the proxyl group is significantly less polar than the doxyl group and b, the ESR spectrum of the proxyl nitroxide is more sensitive to changes in polarity of the medium than that of the doxyl nitroxide.

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References and Notes

(1) Alfred P. Sloan Fellow; NIH RCDA recipient.
(2) J. F. W. Keana and T. D. Lee, *J. Am. Chem. Soc.*, **97**, 1273 (1975).
(3) P. Jost and O. H. Griffith, *Methods Pharmacol.*, **2**, 223 (1972).
(4) See, inter alia, S. Chou, J. A. Nelson, and T. A. Spencer, *J. Org. Chem.*, **39**, 2356 (1974); J. A. Cella, J. A. Kelley, and E. F. Kenehan, *J. Org. Chem.*, **40**, 1860 (1975).
(5) For a review, see B. J. Gaffney, in "Spin Labeling: Theory and Applications", L. J. Berliner, Ed., Academic Press, New York, N.Y., 1975, Chapter 5.
(6) E. Lunt, *Nitro Compd., Proc. Int. Symp.*, 291 (1963); *Chem. Abstr.*, **64**, 676 (1966).
(7) Pyrroline nitrones have also been used as free radical trapping agents, see E. G. Janzen and C. A. Evans, *J. Am. Chem. Soc.*, **95**, 8205 (1973), and M. Iwamura and N. Inamoto, *Bull. Chem. Soc. Jpn.*, **40**, 703 (1967).
(8) R. Bonnett, R. F. C. Brown, V. M. Clark, I. O. Sutherland, and A. Todd, *J. Chem. Soc.*, 2094 (1959).
(9) By analogy to the term "doxyl" we suggest the term "proxyl" when specifying the position of the pyrrolidine nitroxide grouping in a spin labeled molecule.
(10) The tetrahydropyranyl ether nitroxide (*m/e* 452.410) has also been obtained in pure form.
(11) *m/e* 286.131. Prepared by reaction of 1-lithiodecyne with 1,5-dibromopentane.
(12) E. J. Corey and C. U. Kim, *J. Am. Chem. Soc.*, **94**, 7586 (1972).
(13) *m/e* 370.332. Prepared from 7-oxooctadecanol by the method of Keana.[14]
(14) J. F. W. Keana, S. B. Keana, and D. Beetham, *J. Am. Chem. Soc.*, **89**, 3055 (1967).

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Micelle Catalyzed Reactions Are Models of Enzyme Catalyzed Reactions Which Show Positive Homotropic Interactions

Sir:

In attempting to understand the mechanisms by which enzymes catalyze reactions, chemists have expended much effort in the study of catalysis in simpler, chemical systems.[1] Catalysis of reactions within micelles has been studied extensively from this point of view[2,3] since both micelles and enzymes bind substrates in a noncovalent manner. The purpose of this report is to demonstrate an additional similarity of micelle catalysis and enzymatic catalysis: the kinetics of micelle catalyzed reactions are similar to those of many regulatory enzymes in that they show positive homotropic interactions.[4] A simple kinetic description of enzymatic homotropic interactions may be applied to analysis of the kinetics of a vast number of micelle catalyzed reactions.

The rate constants for micelle catalyzed reactions when plotted vs. detergent concentration yield approximately sigmoid-shaped curves; downward sloping sigmoid curves have also been observed for cases in which micelles inhibit reaction. The similarities in shape of these curves to the sigmoid-shaped dependencies of velocity on substrate concentration produced by many regulatory enzymes are striking. The kinetic model commonly used to quantitatively describe the relationship of rate constant to detergent concentration assumes that micelle, D_n, forms a noncovalent complex with substrate, S, before catalysis may take place.[3]

$$\begin{aligned} D_n + S &\overset{K}{\rightleftharpoons} D_nS \\ D_nS &\overset{k_m}{\rightarrow} \text{product} \\ S &\overset{k_0}{\rightarrow} \text{product} \end{aligned} \qquad (1)$$

In this scheme K is the association constant of the micelle-substrate complex, k_m is the rate constant for micelle-catalyzed reaction, and k_0 is the rate constant for reaction in the absence of micelle. The observed rate constant at any concentration of micelle is given by

$$k_{obsd} = \frac{k_0 + k_m K\left(\frac{[D]_{total} - CMC}{n}\right)}{1 + K\left(\frac{[D]_{total} - CMC}{n}\right)} \qquad (2)$$

where CMC is the "critical micelle concentration" which is defined as that concentration of detergent at which micelles first appear, and n is the number of detergent molecules per micelle. This relationship and its equivalents describe reasonably well the dependence of the rates of very many micelle catalyzed reactions, and it has received wide acceptance.

An alternative model[5] postulates that substrate and detergent molecules aggregate to form micelles, D_nS, which may then react to yield product:

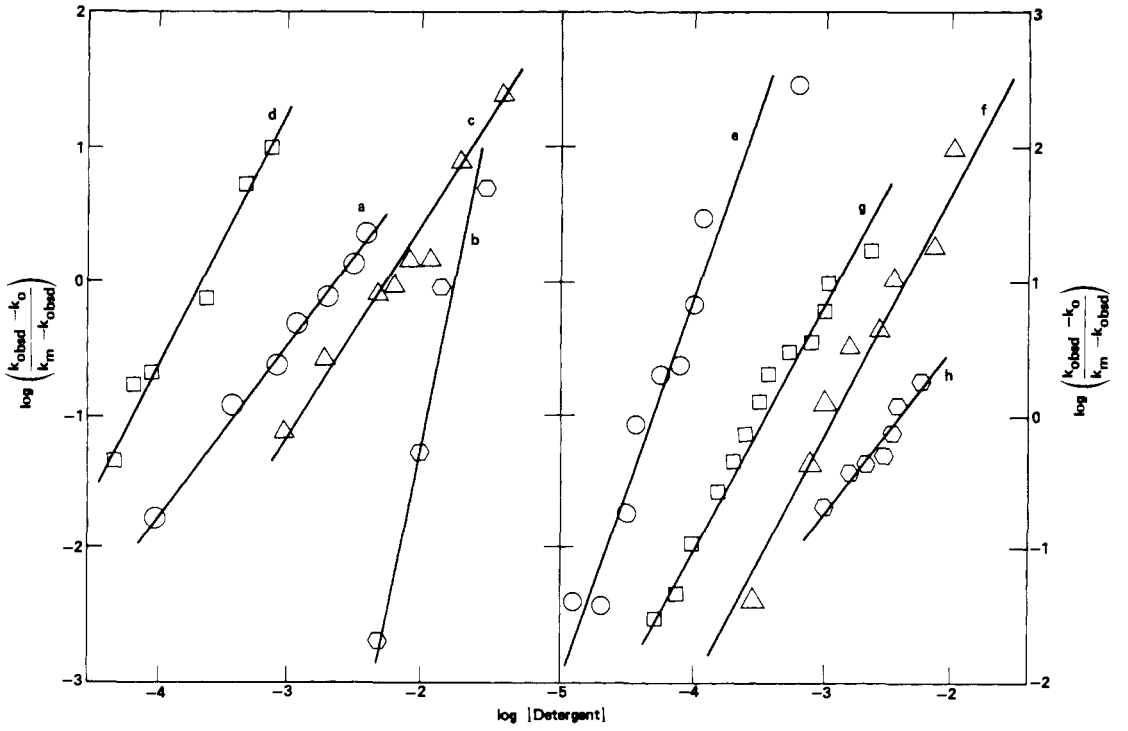

Figure 1. Hill-type plots according to eq 8 of reactions catalyzed by detergents: (a) acid catalyzed hydrolysis of methyl orthobenzoate in sodium hexadecyl sulfate, $n = 1.31$, $(\log K_D)/n = -2.64$, (data from ref 15); (b) acid catalyzed hydrolysis of methyl orthobenzoate in sodium dodecyl sulfonate, $n = 5.0$, $(\log K_D)/n = -1.75$ (data from ref 16); (c) oxidation of benzaldehyde in polyoxyethylene (24) hexadecanol, $n = 1.58$, $(\log K_D)/n = -2.26$ (data from ref 17); (d) decomposition of 1,1-dimethoxy-2,4,6-trinitrocyclohexadienyl ion in the reversed micelle of dodecylammonium benzoate in benzene, $n = 1.90$, $(\log K_D)/n = -3.64$ (data from ref 18); (e) inhibition of hydrolysis of sodium 3-nitro-4-hexanoylbenzenesulfonate in cetyltrimethylammonium bromide, $n = 2.92$, $(\log K_D)/n = -4.32$ (data from ref 5); (f) inhibition of hydrolysis of sodium 3-nitro-4-hexanoylbenzenesulfonate in $HOCH_2CH_2(OCH_2CH_2)_{17}OC_6H_4(CH_2)_{11}CH_3$, $n = 1.82$, $(\log K_D)/n = -3.04$ (data from ref 5); (g) hydrolysis of 2,6-dinitrophenyl phosphate in 2,4-dimethoxyphenylcetyldimethylammonium bromide, $n = 1.87$, $(\log K_D)/n = -3.50$ (data from ref 19); (h) mutarotation of 2,3,4,6-tetramethyl-α-D-glucose in the reversed micelle of dodecylammonium benzoate in benzene, $n = 1.32$, $(\log K_D)/n = -2.5$ (data from ref 20).

$$nD + S \underset{K_D}{\rightleftharpoons} D_nS$$
$$D_nS \xrightarrow{k_m} \text{product} \qquad (3)$$
$$S \xrightarrow{k_0} \text{product}$$

K_D is the dissociation constant of the micelle back to its free components. For this reaction scheme the observed rate constant is expressed as a function of the concentration of detergent, D, by

$$k_{obsd} = \frac{k_m[D]^n + k_0K_D}{K_D + [D]^n} \qquad (4)$$

This formulation has been used infrequently, probably due to the difficulties involved in evaluating the constants involved, especially the exponent n.

In the field of enzyme kinetics numerous models have been proposed to describe the sigmoid dependence of rate on substrate concentration. The simplest of these was proposed by Hill[6] to describe the S-shaped curve for the binding of oxygen to hemoglobin. More recently Atkinson et al.[7] have adapted the Hill model to describe enzymatic reactions. The overall enzyme catalyzed reaction is expressed as

$$E + nS \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES_n$$
$$ES_n \xrightarrow{k_3} \text{product} \qquad (5)$$

The velocity, v, of the enzyme catalyzed reaction (eq 5) as a function of substrate concentration is given by

$$v = \frac{V_{max}[S]^n}{K' + [S]^n} \qquad (6)$$

where v is the observed velocity, V_{max} is the maximum attainable velocity, and $K' = (k_2 + k_3)/k_1$. Equation 6 may be rearranged to yield

$$\log \frac{v}{V_{max} - v} = n \log [S] - \log K' \qquad (7)$$

A plot of $\log [v/(V_{max} - v)]$ vs. $\log [S]$ is linear. The slope of this line is n, and at $\log [v/(V_{max} - v)] = 0$, $\log [S] = (\log K')/n$. While the Hill model has theoretical shortcomings, it also has practical advantages which give it a place in contemporary enzymology.[8,9] Graphical analysis allows simple evaluation of $(\log K')/n$ which is that concentration of substrate at which half maximal velocity is obtained. Also, the constant n is viewed as an "index of cooperativity", a quantitative measure of the degree to which binding of one ligand molecule affects binding of additional ligands.

The alternative micelle catalysis model (eq 3) and the Hill model (eq 5) are described by very similar theoretical rate expressions (eq 4 and 6, respectively). Equation 4, which describes micellar catalysis, can be transformed to a linear form in a manner analogous to that in which eq 7 was generated:

$$\log \frac{k_{obsd} - k_0}{k_m - k_{obsd}} = n \log [D] - \log K_D \qquad (8)$$

According to this equation, a plot of $\log [(k_{obsd} - k_0)/(k_m - k_{obsd})]$ vs. $\log [D]$ for a micelle catalyzed reaction is linear with a slope of n; and at $\log [(k_{obsd} - k_0)/(k_m - k_{obsd})] = 0$, $\log [D] = (\log K_D)/n$. The description of micelle catalyzed reactions given in eq 3, 4, and 8 is of value for the same reasons that the Hill model is useful in describing enzymatic reactions.

Data tabulated in the literature were used to construct plots of $\log [(k_{obsd} - k_0)/(k_m - k_{obsd})]$ vs. $\log$ [detergent]. This

treatment was applied only to the region of detergent concentration in which the initial sigmoid dependence of k_{obsd} on detergent concentration was seen. It did not include the region at very high detergent concentration where k_{obsd} often decreases in bimolecular reactions due to dilution of one reactant in the micellar phase. A number of representative plots are shown in Figure 1; they include various types of reactions using a wide variety of detergents and conditions. The general linearity of these plots indicate that the quantitative expression which describes them (eq 8) describes, at least empirically, a large number of micelle catalyzed reactions.

The slopes of the plots range from approximately 1 to 5. These values are far less than the number of 10 to 100 detergent molecules which are found in micelles[3] and which would be predicted on the basis of eq 3. Such low exponential terms in eq 4 have previously been interpreted as being the number of surfactant molecules per substrate molecule in a catalytically productive pre-micelle.[5] The low values of n and their nonintegral nature may also suggest multiple equilibria in the formation of catalytically productive micelles.

The model described by eq 3 does not require distinct conformational forms of the catalyst which are the basis of the Monod-Wyman-Changeux[10] and the Koshland-Nemethy-Filmer[11] models for enzymatic homotropic interactions. Nevertheless, it is probable that catalytic micelles have different shapes in the absence of and in the presence of substrates, since both ionic and nonionic solutes are known to induce changes in the shapes of micelles.[12] Thus, the kinetics of micellar catalysis could be treated analogously to either of the two enzymatic conformational models,[10,11] although such a treatment would be extremely complex.

An apparent shortcoming of the alternative mechanism of micellar catalysis (eq 3) as a model for enzymatic positive homotropic interactions is that the phenomenon results from cooperativity in association of the subunits of the micelle, the detergent molecules, rather than association of the substrate to the detergent. This apparent shortcoming is reconcilable, at least in a qualitative sense, with a model proposed by Frieden.[13] Frieden has noted that many enzymatic homotropic interactions can be related to the degree of aggregation of a polymeric enzyme. One form of the enzyme, either dissociated or aggregated, would have the greater catalytic activity. He also noted that his model was analogous to the Monod-Wyman-Changeux model,[10] the only difference being that the two conformational forms which exist in equilibrium are monomer and oligomer.

The occurrence of homotropic interactions in enzymatic reactions has often been associated with the fact that enzymes catalyzing these reactions are always composed of subunits. A causal relationship between the association of subunits to form functional aggregates which show homotropic interactions has been inferred.[14] Micellar systems which catalyze chemical reactions are simple models of such systems. The model differs from the enzyme in that the subunit is a simple detergent molecule rather than a complex polypeptide chain. Thus, the causes of positive homotropic interactions in enzymatic and micelle catalyzed reactions are related; they can be traced to self-assembly of subunits in both cases.

References and Notes

(1) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms", Vol. 1 and 2, W.A. Benjamin, New York, N.Y., 1966; W. P. Jencks, "Catalysis in Chemistry and Enzymology", McGraw-Hill, New York, N.Y., 1969; M. L. Bender, "Mechanisms of Homogenous Catalysis from Protons to Proteins", Wiley, New York, N.Y., 1971; T. C. Bruice, in "The Enzymes", 3d ed, Vol. 2, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1970, p 217.
(2) E. H. Cordes and R. B. Dunlap, *Acc. Chem. Res.*, **2**, 329 (1969).
(3) E. J. Fendler and J. H. Fendler, *Adv. Phys. Org. Chem.*, **8**, 271 (1970); J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems", Academic Press, New York, N.Y., 1975.
(4) A homotropic interaction is defined as a stimulation or inhibition of activity by the interaction of additional molecule(s) of the same substance with an enzyme; it describes the phenomenon of sigmoid-shaped dependence of velocity on enzyme concentration. The term "allostery" is often used imprecisely to describe this phenomenon. Allostery means the modification of enzymatic activity resulting from attachment of a substance, either substrate or modifier, at a site other than the catalytic site; it describes a mechanism of stimulation or inhibition of activity. K. J. Laidler and P. S. Bunting, "The Chemical Kinetics of Enzyme Action", 2d ed, Clarendon Press, Oxford, 1973, p 370.
(5) T. C. Bruice, J. Katzhendler, and L. R. Fedor, *J. Am. Chem. Soc.*, **90**, 1333 (1968).
(6) A. V. Hill, *J. Physiol.*, **40**, iv (1910).
(7) D. E. Atkinson, J. A. Hathaway, and E. C. Smith, *J. Biol. Chem.*, **240**, 2682 (1965).
(8) A. Cormick-Bowden and D. E. Koshland, Jr., *J. Mol. Biol.*, **95**, 201 (1975).
(9) D. E. Koshland, Jr., in "The Enzymes", 3d ed, Vol. 1, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1970, p 342.
(10) J. Monod, J. Wyman, and J. P. Changeux, *J. Mol. Biol.*, **12**, 88 (1965).
(11) D. E. Koshland, G. Nemethy, and D. Filmer, *Biochemistry*, **5**, 365 (1966).
(12) C. A. Bunton, "Reaction Kinetics in Micelles", E. H. Cordes, Ed., Plenum Press, New York, N.Y., 1973, p 73.
(13) C. Frieden, *J. Biol. Chem.*, **242**, 4045 (1967).
(14) D. E. Koshland, Jr., and K. E. Neet, *Annu. Rev. Biochem.*, **37**, 359 (1968).
(15) R. B. Dunlap and E. H. Cordes, *J. Am. Chem. Soc.*, **90**, 4395 (1968).
(16) R. B. Dunlap and E. H. Cordes, *J. Phys. Chem.*, **73**, 361 (1969).
(17) A. G. Mitchell and L. S. C. Wan, *J. Pharm. Sci.*, **54**, 699 (1965).
(18) J. H. Fendler, *J. Chem. Soc., Chem. Commun.*, 269 (1972).
(19) C. A. Bunton, L. Robinson, and L. Sepulveda, *J. Org. Chem.*, **35**, 108 (1970).
(20) J. H. Fendler, E. J. Fendler, R. T. Medary, and V. A. Woods, *J. Am. Chem. Soc.*, **94**, 7288 (1972).

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Long-Lived Radical Cations from Mesocyclic Dithioethers

Sir:

We wish to report that long-lived, nonaromatic, sulfur radical cations are formed by treatment of eight- and nine-membered mesocyclic dithioethers with one-electron oxidizing agents such as $Cu(CH_3CN)_4(BF_4)_2$, nitrosyl tetrafluoroborate, $NOBF_4$, or nitrosyl hexafluorophosphate, $NOPF_6$, in either acetonitrile or nitromethane.

The radical cation from 1,5-dithiacyclooctane (DTCO) is usually prepared by mixing solutions of 0.01 M $Cu(CH_3CN)_4(BF_4)_2$[1] and 0.03 M DTCO in dry nitromethane. A yellow colored solution with λ_{max} of 412 nm is formed instantly. At room temperature the solution gives an ESR spectrum consisting of a complex multiplet with a g value of 2.012 and showing no saturation at high microwave power. The ESR spectrum of the radical is observable for at least 72 h at room temperature. This g value and the splitting pattern suggests that the unpaired electron is localized on sulfur rather than carbon and is coupled to the α-hydrogens. The same spectrum is observed when DTCO is allowed to react with 1 mol of solid $NOBF_4$ or $NOPF_6$ in acetonitrile.

Since monothioethers (dimethyl sulfide, tetrahydrothiophene), acyclic dithioethers (2,5-dithiahexane), and mesocyclic dithioethers with six-, seven, and ten-membered rings (1,4-dithiane, 1,4-dithiacycloheptane, and 1,6-dithiacyclodecane) *do not give stable radicals* under conditions of the reaction, the transannular sulfur atom in the eight- and nine-membered mesocyclic rings appears to be responsible for the stability of the radical.

Aliphatic sulfur radical cations have been reported recently by treatment of some thioethers (e.g., tetrahydrothiophene, dimethyl sulfide) either with hydroxyl radicals generated by Ti^{III} and H_2O_2 in a flow system[2] or by pulse radiolysis in water.[3] During the studies it was found that when thioethers are oxidized with hydroxyl radicals, a monomeric cation radical, $R_2S\cdot^+$ is not observed. The initially formed monomer apparently reacts further with a neutral thioether molecule to form a dimeric radical cation, $R_2\dot{S}\text{-}S\text{-}R_2^+$, which is observed