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A REMARKABLE ENHANCEMENT OF THE RATE OF ESTER THIOLYSIS BY SYNTHETIC AMPHIPHILE VESICLES

IOLANDA M. CUCCOVIA, FRANK H. QUINA and HERNAN CHAIMOVICH
Group for Interfacial Studies (GIST), Instituto de Química, Universidade de São Paulo, Caixa Postal 20780, São Paulo, Brasil

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Abstract—Cationic surfactant vesicles accelerate the rate of thiolysis of *p*-nitrophenyl octanoate by *n*-heptylmercaptan by several million fold in the pH range from 4 to 6, providing an efficient system for ester thiolysis in aqueous solution that is functional even at pH4, i.e. more than 6 pH units below the pKa of the SH group. Analysis of the data in terms of an ion exchange formalism implies that this rate acceleration is due primarily to concentration of the reagents in the dimensionally-restricted environment provided by the vesicle, coupled with small contributions from enhanced dissociation and reactivity of the nucleophile at the vesicle surface(s).

Recently, there has been an increasing interest in the solution properties of synthetic amphiphiles having a hydrophobic–hydrophilic balance similar to that of natural lipids.[1,2] Although the polar groups of these synthetic amphiphiles bear little[3] or no[4] structural similarity to those of natural lipids, they have been shown to form a variety of lipid-like structures[1] in aqueous dispersions, including both single[5,6] and multibilayer[6,7] vesicles and other types of bilayers.[8] The fact that the chemical nature of these amphiphiles can be readily varied provides a unique opportunity to investigate the interrelationships between the structure of the monomer and the properties and architecture of the resultant aggregates. These features also make synthetic-amphiphile aggregates an extremely attractive model system[9–11] for the study of chemical reactivity in well-defined, dimensionally-restricted environments such as those prevailing at the active sites of enzymes and in biological membranes.

In this work, we report that the thiolysis of *p*-nitrophenyl octanoate (NPO) by *n*-heptyl mercaptan (HM) at pH 4 is accelerated by more than 10^6 fold in the presence of vesicles formed from dimethyl di-(*n*-alkyl) ammonium chloride. Analysis of this marked rate acceleration in terms of an ion-exchange formalism[12] permits estimation of the relative contributions of local concentration, local pH effects, and changes in intrinsic reactivity to the overall rate enhancement.

MATERIALS AND METHODS

NPO was kindly furnished by Dr. O. A. El Seoud.† Dimethyldi-(*n*-alkyl) ammonium chloride (DODAC)‡ was purified and analyzed as described previously.[10] HM§ was distilled *in vacuo* (b.p. 37–38°C, 1 mm Hg) and stored at −18° under argon. All other reagents were analytical grade or superior and solutions were prepared in deionized water, doubly distilled in glass, which had been freshly boiled and allowed to cool under an argon atmosphere.

Kinetic measurements were carried out at 30.0° with a Beckman M25 spectrophotometer. The thiolysis of NPO by HM was followed by monitoring the appearance of the *p*-nitrophenoxide ion at 405 nm (or of *p*-nitrophenol at 330 nm at the lower pHs). Rate constants were calculated as previously described[10] from $\log(A_\infty - A_t)$ vs time plots, which were linear for at least three half-lives in all cases. pH was measured with a Metrhom Herisau E 388 compensator equipped with a semimicro combination electrode (Beckman Inc.) and calibrated against standard reference buffers (Beckman Inc.). DODAC vesicles were prepared as previously described[10] by injection of 1.0 ml of an 0.2 M ethanolic soln of DODAC into 19.0 ml of the appropriate buffer thermostatted at 50°. The soln was stirred vigorously throughout the injection procedure, which required *ca.* 2 min.

Gel filtration of DODAC vesicles (0.50 ml aliquot) was performed on Sephadex G-25 (Pharmacia Fine Chemicals, AB) columns (0.8 × 47 cm) equilibrated with appropriate buffers and eluted with the equilibrating buffer at 4.8 ml/hr; 1.0 ml aliquots were collected. Chloride ion concentrations were determined by titration[13] using a Beckman Spinco 153 Microtitrator and vesicle concentration was estimated by measuring the apparent turbidity at 240 nm. The apparent pK (pKap) of HM was calculated from the relationship $pKap = pH + \log[(HM^-)/(HM_T - HM^-)]$ where HM_T is the total concentration of added HM. The concentration of mercaptide ion (HM^-) was determined at 230 nm[14] as a function of DODAC concentration at constant pH[12,14] and HM_T was determined by titration with 5,5′-dithiobis (*p*-nitrobenzoic acid).[15]

RESULTS

The integrity of DODAC vesicles, prepared by direct injection of concentrated (0.2 M) ethanolic solutions of the DODAC into the appropriate aqueous buffers, was verified by Sephadex G-25 gel filtration.[10] Filtration of DODAC vesicles prepared and filtered in borate buffers results in incomplete borate/chloride ion exchange, most of the chloride eluting at or near V_0.[10] In contrast, when DODAC vesicles are prepared and filtered in acetate buffers, all of the amphiphile-derived chloride elutes at the internal (included) volume of the Sephadex G-25 column, indicating complete displacement of chloride due to ion exchange during the preparation–filtration sequence (Fig. 1).

The influence of unfiltered DODAC vesicles on the pKap of HM is shown in Fig. 2. The effects of unfiltered DODAC vesicles, prepared in acetate buffers, on the apparent first order rate constant (k_ψ) for thiolysis of NPO by HM are shown in Fig. 3.

The maximum increase in the apparent first order rate constant in the presence of vesicles was calculated from

†The Instituto de Química of the Universidade de São Paulo.
‡Herquat 2HT; 85% *n*-C_{18} and 15% *n*-C_{16}, Herga Ind. Quim., Brasil.
§The Connecticut Hard Rubber Co. Chemical Division.

Table 1. Effect of DODAC vesicles on the rate of thiolysis of *p*-nitrophenyl octanoate by *n*-heptylmercaptan

pH	DODAC (M)x10^5	k_ψ^{max} (s^{-1})x10^3	k_ψ^o (s^{-1})x10^9	R_{max} (k_ψ^{max}/k_ψ^o)
4.00	2.3	2.3	0.30	7.7 x 10^6
4.35	6.0	3.9	0.67	5.8 x 10^6
4.60	5.4	4.7	1.20	3.9 x 10^6
5.00	4.7	8.0	3.00	2.7 x 10^6
5.35	8.3	18.4	6.70	2.7 x 10^6
5.60	10.0	27.0	11.90	2.3 x 10^6

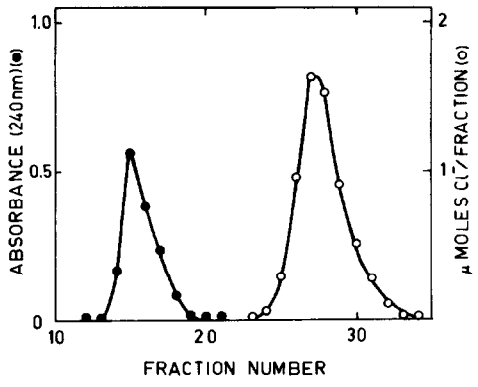

Fig. 1. Gel filtration of DODAC vesicles prepared in 0.005 M Na^+-Acetate buffer, pH 5.30; (○) chloride; (●) Abs_{240}.

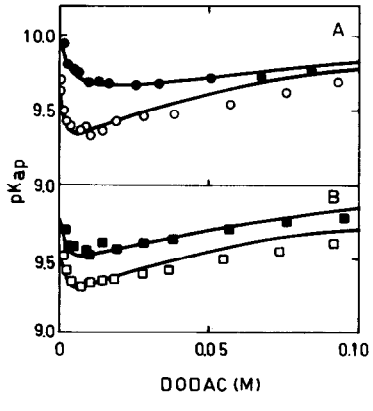

Fig. 2. Effect of DODAC vesicles on the apparent pKa (pKap) of *n*-heptylmercaptan (HM). The pKap of HM (5×10^{-5} M) was determined in the same buffer used to prepare the vesicles (see Materials and Methods). Vesicles were prepared in : A. (●) Tris . HCl 0.02 M, pH 9.1, and (○) Borate (Na^+) 0.01 M, pH 9.3; B. (□) Tris. Acetate 0.02 M, pH 9.05, and (■) Tris. Acetate 0.04 M pH 9.05. All curves are calculated (see Discussion).

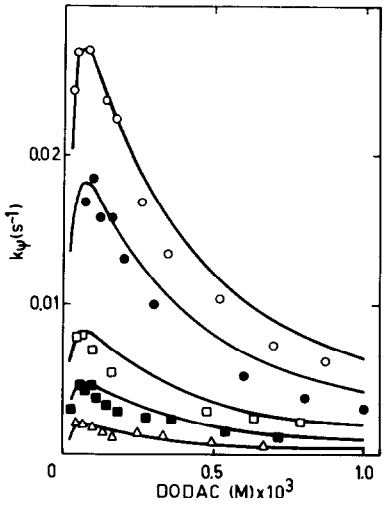

Fig. 3. Effect of DODAC vesicles on the thiolysis of *p*-nitrophenyloctanoate (NPO) by *n*-heptylmercaptan (HM). Rate constants were measured (see Materials and Methods) using 5×10^{-6} M NPO and 5×10^{-5} M HM in Na^+ Acetate buffers. (△) pH 4.0; (■) pH 4.60; (□) pH 5.0; (●) pH 5.35; (○) pH 5.60. All curves are calculated (see Discussion).

the relationship:

$$R_{max} = k_\psi^{max}/k_\psi^{\,0} \qquad (1)$$

where k_ψ^{max} is the maximum value of k_ψ at each pH (Fig. 3). The corresponding apparent first order rate constants in the absence of vesicles ($k_\psi^{\,0}$) were calculated from

$$k_\psi^{\,0} = k_2^{\,0} \times HM_T \times \frac{10^{(pH-pKa)}}{1+10^{(pH-pKa)}} \qquad (2)$$

using the measured values of $k_2^{\,0} = 30\ M^{-1}\ s^{-1}$ for the second order rate constant of thiolysis in the aqueous phase, 10.70 for the pKa of HM[14,16] and the total concentration of HM (HM_T). The relevant data are summarized in Table 1.

DISCUSSION

The mere demonstration of a million fold increase in the apparent constants in a non-enzymatic model system sheds little light on either the origin of the rate increase or its relationship to the rates observed in enzyme-catalysis. In the present case, the overall rate acceleration is so large relative to those observed in other model systems that it becomes essential to understand the various factors that contribute to this rate enhancement.

The existence of a minimum and the effect of added acetate on the pKap vs DODAC profiles (Fig. 2) are completely analogous to the effects of cationic micelles on the dissociation of HM and other weak acids.[12,14] In the case of micelles, the pKap profiles can be analyzed quantitatively using an ion-exchange formalism which describes this complex pattern in terms of the distribution of HM between the aqueous and micellar phases and local pH effects (ion-exchange) at the micellar surface.[12,14] The applicability of these concepts of ion exchange in the DODAC system follows directly from the gel filtration data, which provide unequivocal evidence for Cl^-/borate and Cl^-/acetate ion exchange at the DODAC vesicle surface(s). Moreover, the observation of complete displacement of chloride by acetate (Fig. 1) implies that, in this case, ion exchange occurs at both the inner and outer surfaces of the vesicle. Thus, by analogy to the micellar system, the effect of unfiltered DODAC vesicles on the pKap of HM should be described[14] by the relationship:

$$Kap = K_a \frac{1 + K_{HM^-/Cl}\dfrac{Cl_b}{Cl_f}}{1 + K_{HM}|DODAC|} \qquad (3)$$

where Ka is the acid dissociation constant of HM in water, K_{HM} is the distribution constant of HM between the water and vesicle phases, and $K_{HM^-/Cl}$ is the weighted selectivity coefficient for mercaptide ion/chloride ion exchange at the outer and inner vesicle surfaces. In the presence of an "ideal" buffer such as Tris-HCl,[17] the analytical concentrations of bound and free chloride ions, Cl_b and Cl_f respectively, can be calculated from expressions derived previously.[12,20]

$$Cl_b = (1 - \alpha)|DODAC| - OH_b \qquad (4)$$

$$Cl_f = \alpha|DODAC| + Cl_{AD} + OH_b \qquad (5)$$

where Cl_{AD} is the concentration of added chloride and OH_b the concentration of bound hydroxide ion. The value for the selectivity coefficient for hydroxide/chloride exchange ($K_{OH/Cl} = 0.13$) was taken to be that derived for hexadecyltrimethylammonium micelles.[14,17] Using a ratio of $K_{HM^-/Cl}/K_{HM}$ of 0.03, a value close to that obtained in micellar systems[14] and a K_{HM} of 3700,[14] the pKap profile obtained in Tris-HCl (Fig. 2a) could be fitted with a constant value of 0.16 for the parameter α, which reflects the apparent degree of counterion dissociation at both the inner and outer vesicle surfaces. The pKap vs DODAC profiles depicted in Fig. 2 could all be reproduced by simply taking into account the effects of borate/Cl and acetate/Cl exchange on Cl_b/Cl_f using the corresponding ion exchange selectivity coefficients determined for cationic micelles.[14,18,19]

Although the absolute values of the parameters employed in the curve fitting procedure represent only estimates, the fact remains that the experimental results for the effects of vesicles on pKap can be fitted remarkably well with a micelle-type ion exchange formalism. Hence, we are led to conclude that the vesicle surface(s) are micelle-like with respect to counterion exchange, the "small" effect of DODAC vesicles on the dissociation of HM being attributable to distribution of HM between the aqueous and vesicle "phases" and ion-exchange effects on the local pH at the vesicle surface(s).

The overall reaction rate for the thiolysis of NPO by HM in the presence of DODAC vesicles is the sum of the rates of reaction in the aqueous phase (governed by the rate constant in the aqueous phase and the analytical concentrations of the free reagents) and in the vesicle "phase" (governed by the rate constant and the *local* concentrations of reagents in this phase). Since the volume fraction occupied by the vesicles is very small[11] and both NPO and HM can be safely assumed to partition in favor of the vesicle bilayer[14] the *local* reagent concentration in the vesicles can be several orders of magnitude higher than those in the bulk aqueous phase. For example, taking the partial molar volume ($\bar{V}$) of DODAC to be 0.44 L/M,[11] the volume fraction occupied by the vesicles (excluding the internal aqueous compartment) at 10^{-4} M DODAC would be 4.4×10^{-5}.

Thus, for a bimolecular reaction between two *totally* vesicle-incorporated substrates at 10^{-4} M DODAC, one would expect a 5×10^8 fold increase in reaction velocity (or a 5×10^4 fold increase in k_ψ) on the basis of local substrate concentration effects alone.

Considering, in addition to substrate distribution, the effects of local pH on the dissociation of HM, it can be shown[12,14] that:

$$k_\psi = (HM)_T K_a \times \frac{(k_2^v/\bar{V})K_S K_{HM^-/Cl}\dfrac{Cl_b}{Cl_f} + k_2^0}{(1 + K_{HM}|DODAC|)(H^+ + Kap)(1 + K_S|DODAC|)} \qquad (6)$$

Using the same parameter values derived from the analysis of the effect of DODAC vesicles on pKap and a value of K_S ($4 \times 10^4\ M^{-1}$), approximately equal to that for NPO in CTAB,[14] the best fit value for $k_2^v/\bar{V}$ was found to be $1095\ s^{-1}$ at all the pH's studied. The increase in external pH produces an increase in the local concentration of bound hydroxide ion at the vesicle interface, and a corresponding increase in k_ψ and k_ψ^{max} due to enhanced dissociation of HM (Fig. 3, Table 1). However, at constant buffer concentration, the increase in pH is accompanied by an increase in the acetate concentration enhancing the relative displacement of bound counterions (including ^-OH) by acetate from the vesicle surface(s). The decrease in R_{max} with pH (Table 1) and the non-unity slope (0.75 at 1×10^{-4} M DODAC) of a log k_ψ vs pH function (data from Fig. 3) are thus nicely accounted for by Eq. 6, via the Cl_b/Cl_f term, on the basis of local pH effects[17] (i.e. ion-exchange at the vesicle interface(s)).

An absolute value for the apparent intrinsic reactivity[17] of the mercaptide ion for the thiolysis reaction in the vesicles (k_2^v) can be calculated from an estimate of $\bar{V}$ and the value of $k_2^v/\bar{V}$. Thus, depending on the value

selected for $\bar{V}$ (ranging from 0.37 to 0.44 L M^{-1}),[10,11] k_2^v varies from 405 s^{-1} M^{-1} to 481 s^{-1} M^{-1}. Compared to the value of $k_2^0 = 30\ s^{-1}\ M^{-1}$, these k_2^v values are indicative of only a moderate increase in the intrinsic reactivity (*ca* 15 fold) of the mercaptide ion in the DODAC vesicle implying that the main acceleration factor is indeed a local concentration effect.

Although the relationship between rate accelerations obtained in amphiphile aggregates and kinetic data for enzymes is not straightforward,[a] the results of the present work bear directly on the role of entropic factors in enzyme catalysis.[20] Thus, the analysis of the kinetic data for the present model system demonstrates that, even in the case of relatively non-specific substrate binding, quite large rate enhancements can arise as a result of concentration of the components of a bimolecular reaction in a dimensionally restricted environment.

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[a]See ref. 20 for a cogent description of the difficulties involved.