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EFFECT OF DIALKYLDIMETHYLAMMONIUM VESICLES ON THE THIOLYSIS OF p-NITROPHENYL ACETATE

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SUMMARY: Cationic surfactant vesicles, prepared by an alcohol injection technique and characterized by gel filtration, are extremely effective at increasing the rate of ester thiolysis at near neutral pH.

We and others have recently demonstrated that synthetic long-chain dialkyl amphiphiles preferentially form positively[1] and negatively[2] charged vesicles upon sonification in aqueous media. Since, unlike natural phospholipids[3], these vesicles are chemically stable, they are unique models for the study of chemical reactivity in membranes. In our initial attempts to study the thiolysis of p-nitrophenyl acetate (NPA) by n-heptylmercaptan (C_7SH) in the presence of dialkyldimethylammonium (Herquat)[4] vesicles, we found that vesicles preparations obtained by sonication contained probe-derived contaminants capable of catalyzing the oxidation of C_7SH. We therefore investigated the possibility of obtaining these vesicles by the alcohol injection method[5]. Vesicles are indeed obtained from Herquat[4] by this technique[5]. When prepared in the presence of boric acid--borax buffer (9×10^{-3}M), the vesicle preparations is stable for at least 24 hrs. The existance of vesicles was confirmed by gel filtration on Sephadex G-25 (fine) using three independent criteria: (1) The gel filtration data in Fig.1A show that our Herquat vesicles are capable of entrapping (^{14}C)-glucose to the extent of 8.8%, a value which compares favorably with that reported for analogous[1] vesicles. The stability of the vesicles under these conditions is indicated by the retention of the incorporated glucose upon refiltration of the vesicle preparation (Fig.1B). (2) Gel filtration of a vesicle preparation labelled with the water-insoluble fluorescent probe methyl 4-(1-pyrene)-butyrate, which should be totally incorporated in the bilayer phase[6] resulted in a very sharp fluorescence peak in the void volume of the column (Fig.1C). (3) Preliminary kinetic studies, with ulfiltered vesicle dispersions, demonstrated that the thiolysis of NPA by (exess) C_7SH is pseudo first-order under all conditions and that the rate increases with Herquat concentration up to ca. 0.4 x 10^{-3}M. That the observed rate acceleration is indeed attributable exclusively to vesicles is patent from the agreement between the observed rate acceleration profile for the gel filtration aliquots (Fig.1D) and the profiles for both entrapped (^{14}C)-glucose and the fluorescent pyrene derivative.

In contrast to these three criteria for the vesicle elution profile, the chloride ion profile was found to be significantly broader on the trailing edge (Fig.1E). The sensitivity of this broadening to the ionic content of the medium leads us to conclude that this tailing is related to ion exchange[7] at the vesicle surface(s).

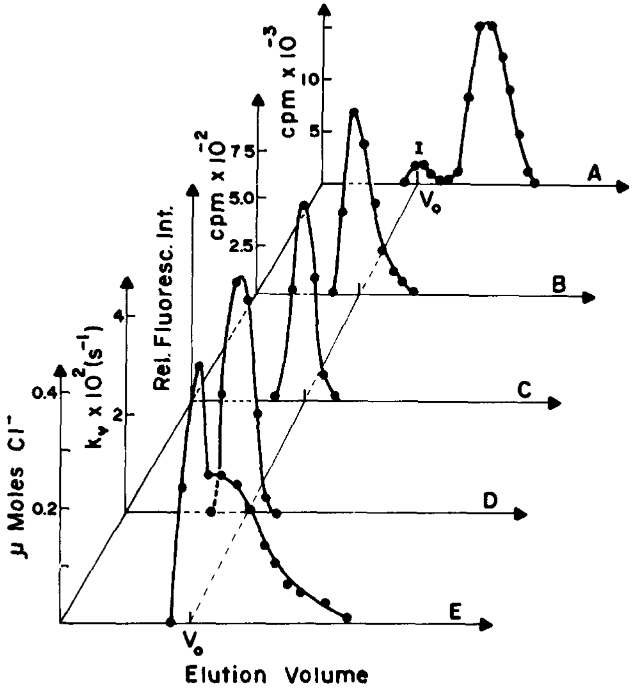

Fig.1 - Gel filtration of Herquat vesicles prepared by injection (9x10^{-3}M borate buffer, pH 7.6). (A) Separation of vesicle-entrapped from free (^{14}C)-glucose; (B) Refiltration of an aliquot of peak I of A; (C) Relative fluorescence intensity of vesicle-incorporated methyl-4-(1-pyrene)-butyrate; (D) Effect of column aliquots on the relative rate of thiolysis of NPA by n-heptanethiol; (E) Chloride ion profile.

Having thus shown that vesicles are present and are the only source of the observed rate increase, we examined the amphiphile dependence of the thiolysis of NPA by C_7SH (Fig.2). There is a distinct rate maximum at 0.5 x 10^{-3}M Herquat with a smooth fall off in rate at higher amphiphile concentrations, the maximum rate increase of 7 x 10^4 in the presence of Herquat vesicles at pH 7.6 being truly remarkable. In contrast, the corresponding maximum rate increase in the presence of CTAB (2 x 10^{-3}M) is only 4.0 x 10^3 under the same conditions[8].

This remarkable rate enhacement may reflect the mutual interplay of interfacial effects on the apparent pK of C_7SH, the binding of the resultant mercaptide and NPA to the vesicle and (possibly) intrinsic effects on nucleophilic reactivity at the vesicular interface(s). Indeed Herquat (5×10^{-4}M) vesicles have been found to decrease the pK_{ap} of C_7SH from 10.7[9] to 9.45. Quantitative studies of this system, envisaging the dissection of the relative roles of the above factors, are in progress.

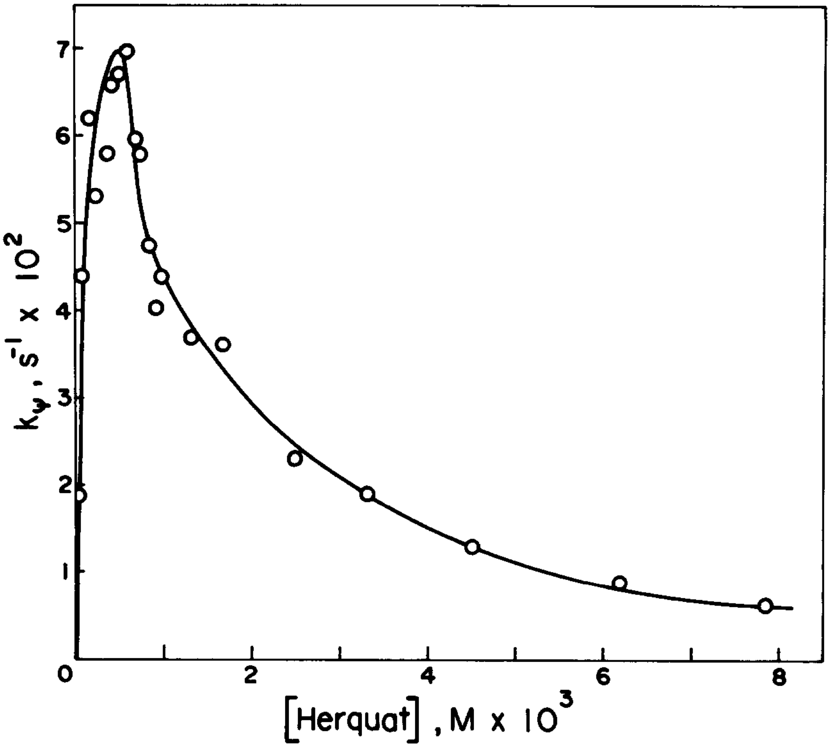

Fig.2 - Effect of Herquat vesicles on the rate of thiolysis of p-nitrophenyl acetate. An appropriate aliquot of Herquat vesicles (prepared by injection of <u>ca.</u> 1 mL of a 0.2 M ethanol solution of Herquat into 19 mL of 1×10^{-2}M boric acid--borax buffer pH 7.6) diluted to 2.5 mL with the same buffer (1×10^{-2} M, pH 7.6) and temperature equilibrated in a Beckman 25 spectrophotometer. 15 µL of a solution of n-heptanethiol (8×10^{-3}M in ethanol) were added and the reaction started by addition of 10 µL of a CH_3CN solution of NPA (1.25×10^{-3}M). Reaction was followed at 405 nm and apparent first-order rate constants were calculated as described[10]. k_ψ for the uncatalyzed thiolysis of NPA by C_7SH under the same conditions is 2.4×10^{-6} s^{-1} [10].

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(4) Herquat 2HT-75, a tradename product of Herga Indũstria Quĩmica S.A., Rio de Janeiro, Brasil, is provided as a mixture of dialkyl (75% C_{18}: 25% C_{16}) dimethylammonium chloride. This material was purified by vacuum drying, Soxhlet extraction with ether, and recystalization (6x) from acetone. Vapor phase chromatographic analysis (using appropriate standards) of both the resultant 1-olefins and the N,N-dimethyl-N-alkylamines following Hofmann degradation of the purified Herquat indicated 85% n-C_{18} and 15% n-C_{16} with a trace of n-C_{14}. Similarly, mass-spectral analysis provided a C_{18}:C_{16} ratio of 86:14, as well as a dialkyl chain distribution of 75% C_{18}/C_{18}, 23% C_{18}/C_{16} and 2% C_{16}/C_{16}.

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