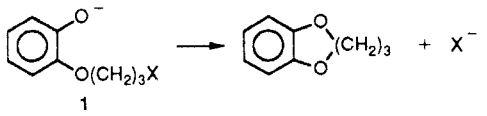
is supported by micellar rate enhancements of cyclization of **1**,



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which is mechanistically an intramolecular S_N2 reaction. With X = Br rate constants in CTACl or CTABr are larger than that in water by a factor of 1.8, but this factor increases to 29 for reaction of X = I in $C_{16}H_{33}NBu_3Br$.[24]

There is a marked difference between the results for micellar reactions of MeONs with Cl^- and Br^-. The Langmuir parameters for micellar binding of Cl^-, derived kinetically and by NMR line widths, are very similar (Table I), but for Br^- the kinetic values are considerably larger than those from NMR line width.[11,12] This difference suggested that NMR line widths sensed largely Br^- that was very close to a cationic headgroup and the kinetics sensed not only these ions but also those that are less tightly bound to the micellar surface. This question will be discussed more fully on related work on the effect of headgroup size on interactions of Cl^- with cationic micelles.[11b]

Experimental Section

Materials. Preparation and purification of MeONs and CTACl have been described.[8b] The other surfactants were obtained by heating alkyl chloride (0.3 mol) with aqueous 4 M trimethylamine (0.5 mol) in 150 mL of EtOH at reflux, generally for 3-4 h. After solvent evaporation, the surfactants were recrystallized two or three times from EtOAc with a small amount of EtOH. The sulfate surfactants were obtained from the corresponding chlorides by treatment with stoichiometric Ag_2SO_4 in EtOH and recrystallization from EtOAc with a small amount of EtOH. The methanesulfonates were obtained from the chlorides by ion exchange using Dowex cationic resin MSA-1 in the methanesulfonate form and purification as above.

Critical Micelle Concentrations. Values of the cmc were determined by surface tension measurements and agreed with available literature data.[25] There were no minima in the surface tension plots.

Kinetics. Reactions were followed spectrophotometrically at 326 nm. MeONs was added in 20 μL of MeCN to 3 mL of reaction solution at 25 °C, so that [MeONs] = 10^{-4} M. The first-order rate constants, $k\psi$ (s^{-1}), for reaction in CTACl agreed with earlier values.[8b,10]

NMR Spectroscopy. The ^{35}Cl line widths were measured on a GE-300MHz FT-NMR spectrometer at 29.445 MHz by using a one-pulse sequence. Typical settings for FT parameters were flip angle 30-45°, delay 1 s, and spectral width 4 kHz. The memory block for storage of the FID with 4K and the resolution was better than 1 Hz. The line width at half-height was calculated by using the Lorenzian fit subroutine. For exponential multiplication a line broadening of 1 Hz was used. The aqueous surfactant or NaCl solution contained 20 vol % D_2O as lock. At this concentration the deuterium isotopic effect is negligible. The simulations were unaffected by a 2-fold variation in the line width in water.

Calculations. The fit of experimental data has been made using a modified program designed initially for fitting of kinetic data.[26]

Acknowledgment. Support by the National Science Foundation (Organic Chemical Dynamics Program) and the Aluminum Company of America is gratefully acknowledged. We are grateful to Dr. Ata Shirazi for his help with the NMR measurements.

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Micellar Effects upon Rates of S_N2 Reactions of Chloride Ion. 2. Effects of Cationic Headgroups

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Observed first-order rate constants for reaction of methyl naphthalene-2-sulfonate with Cl^- in micelles of cetyltrialkylammonium chlorides ($C_{16}H_{33}NR_3Cl$, R = Me, Et, *n*-Pr, *n*-Bu, *n*-Pe) increase monotonically with increasing concentrations of surfactant and Cl^- and tend to limiting values. These variations can be fitted to a model that describes micelle–ion interactions in terms of Langmuir isotherms. The binding parameters, K'_{Cl}, decrease with increasing headgroup bulk, but second-order rate constants at the surface increase. The value of K'_{Cl} is higher with a quinuclidinium as compared with a N^+R_3 headgroup, but the second-order rate constant is similar to that for R = Et. Values of K'_{Cl} are high with hydroxyethyl head groups N^+R_3 = $N^+Me_2CH_2CH_2OH$ or $N^+(CH_2CH_2OH)_3$, but these groups decrease second-order rate constants. Values of K'_{Cl} estimated from ^{35}Cl NMR line widths agree with the kinetic values except with the $N^+(CH_2CH_2OH)_3$ headgroup for which the kinetic values are much higher. The effect of headgroup bulk upon the spontaneous reaction with water was examined by using sulfate as the surfactant counterion. Trends in reactivity are very similar for reactions of H_2O and Cl^-.

The hypothesis that micelles speed bimolecular reactions merely by bringing reactants together and that calculated second-order rate constants at micellar surfaces are very similar[2] is an oversimplification. Reactivities of counterions depend on their solvation shell, which may be perturbed by interaction with the micelle.[3,4]

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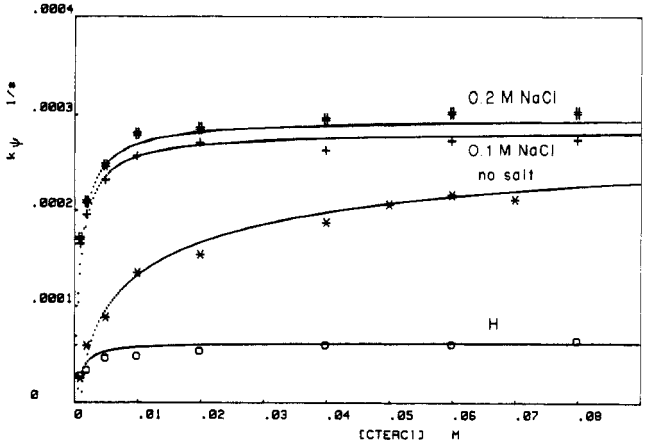


Figure 1. First-order rate constants of reaction of MeONs with Cl^- in CTEACl at indicated [NaCl] and of hydrolysis in $CTEA(SO_4)_{1/2}$ (H).

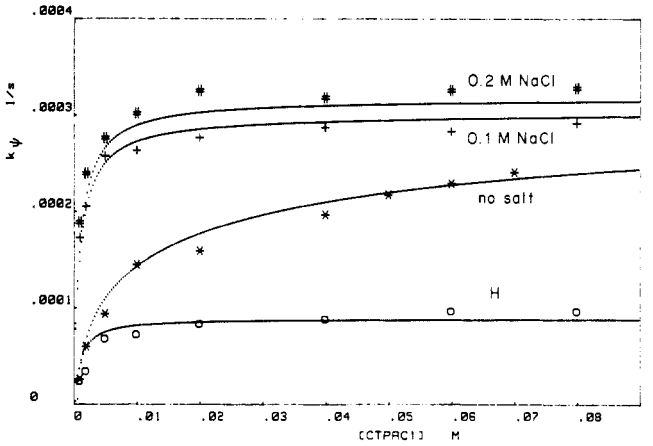
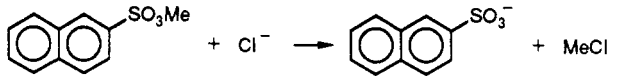


Figure 2. First-order rate constants of reaction of MeONs with Cl^- in CTPACl at indicated [NaCl] and of hydrolysis in $CTPA(SO_4)_{1/2}$ (H).

In the preceding paper we discussed the influence of the length of alkyl tails of alkyltrimethylammonium chlorides on ion–micelle interactions and hydration of Cl^- and its reaction with methyl naphthalene-2-sulfonate (MeONs).[5]



MeONs

The properties are related to the degree of penetration of Cl^- between the cationic surfactant headgroups, and it is useful to examine effects of headgroup size and structure for hexadecyl (cetyl) surfactants.

Fractional micellar ionization, α,[2b-e] increases, i.e., affinity of micelles for counterions decreases,[3,6] with increasing bulk of alkyl headgroups in the series Me < Et < *n*-Pr < *n*-Bu. However, overall rate constants of reactions of MeONs with Br^- and Cl^- increase in the same sequence.[3] Dependence of the NMR line width of Br^- on added NaBr and the headgroup structure show that the reactivity increase is related to disruption of the hydration of Br^- as well as to interaction of the naphthalene π-system of MeONs with the cationic headgroups. The fractional dissociation of Br^-, estimated by NMR, is higher than that estimated kinetically.[3] There are various ways of defining counterion concentration in a micellar pseudophase, and different experimental methods give different results.[7] Chemical reactivity senses reactive ions that can readily interact with micellar-bound substrate, but NMR line width senses ions whose solvation shell is disrupted by interaction with micellar headgroups.[3]

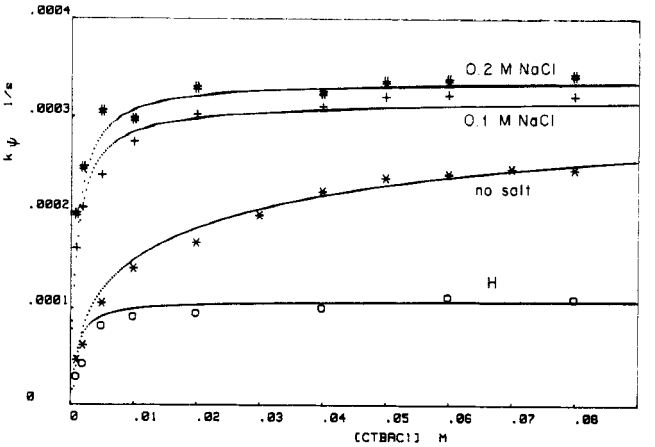


Figure 3. First-order rate constants of reaction of MeONs with Cl^- in CTBACl at indicated [NaCl] and of hydrolysis in $CTBA(SO_4)_{1/2}$ (H).

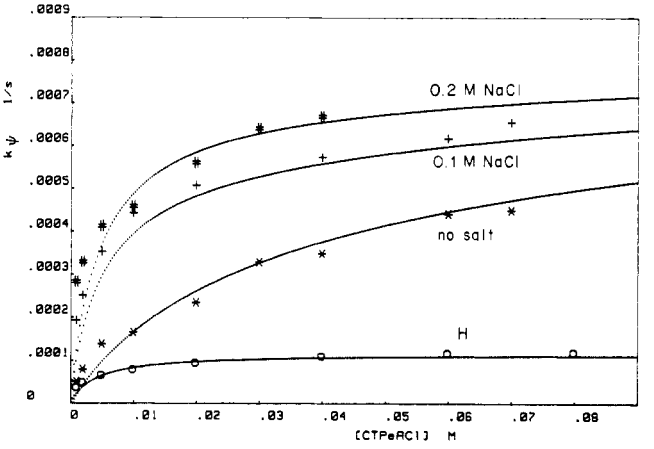


Figure 4. First-order rate constants of reaction of MeONs with Cl^- in CTPeACl at indicated [NaCl] and of hydrolysis in $CTPeA(SO_4)_{1/2}$ (H).

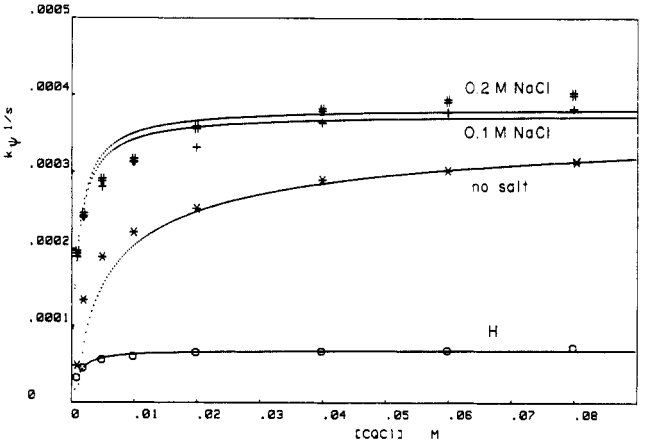


Figure 5. First-order rate constants of reaction of MeONs with Cl^- in CQCl at indicated [NaCl] and of hydrolysis in $CQ(SO_4)_{1/2}$ (H).

We extended results on reaction of MeONs in cetyltrialkylammonium chlorides[3,8] (alkyl = Me (CTACl), Et (CTEACl), *n*-Pr (CTPACl), and *n*-Bu (CTBACl)) to a study of hydrolysis in the

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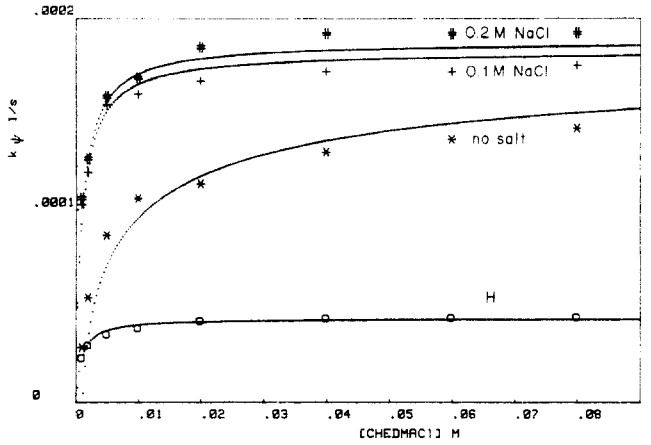

Figure 6. First-order rate constants of reaction of MeONs with Cl^- in CDMHEACl at indicated [NaCl] and of hydrolysis in CDMHEA-$(SO_4)_{1/2}$ (H).

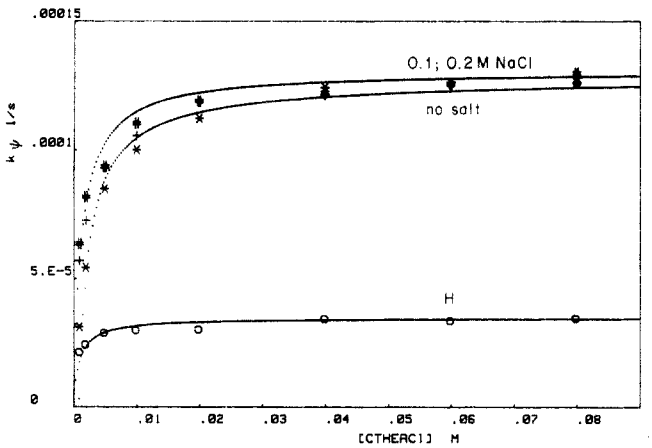

Figure 7. First-order rate constants of reaction of MeONs with Cl^- in CTHEACl at indicated [NaCl] and of hydrolysis in CTHEA$(SO_4)_{1/2}$ (H).

corresponding sulfates. We also examined reactions in C_{16} surfactants with other headgroups, i.e., tripentyl (CTPEACl), dimethyl-2-(hydroxyethyl) (CDMHEACl), trihydroxyethyl (THEACl), and quinuclidinyl (CQCl). We measured dependence of ^{35}Cl line width on surfactant and added salt concentration for all the surfactants. Corresponding data for surfactants with different hydrophobic alkyl groups are in ref 5.

Results

Kinetics. First-order rate constants, $k\psi$, of nucleophilic attack of H_2O and Cl^- increase with [surfactant] and added [NaCl] and reach limiting values (Figures 1–7). These limiting values increase in the sequence Me < Et < *n*-Pr < *n*-Bu < *n*-Pe, similar to that for reaction of Br^-.[3,5] Except for CTHEACl, limiting rate constants are reached only at relatively high [surfactant] and added NaCl speeds reaction, so it appears that the micelles are not saturated with Cl^-.

Rate constant–surfactant profiles can be fitted as in the preceding paper.[5] Values of $k\psi$ for hydrolysis follow[2,9]

$$k\psi = (k_W^\circ + k_M^\circ K_s[D_n])/(1 + K_s[D_n]) \quad (1)$$

The first-order rate constant for reaction of Cl^-, $k\psi^c$ corrected for the reaction with water, follows

$$k\psi^c = (k_W[Cl_W^-] + k_M K_s[Cl_M^-])/(1 + K_s[D_n]) \quad (2)$$

In eqs 1 and 2 subscripts M and W indicate micellar and aqueous pseudophases respectively, superscripts o indicate reaction with water, k_w (M^{-1} s^{-1}) is the second-order rate constant for reaction with Cl^- in water, and k_M (s^{-1}) is that in the micellar pseudophase with concentration of Cl^- written as a mole ratio to micellar headgroups.[3,5] The substrate binding constant, K_s, is defined in terms of micellized surfactant, with $[D_n] = [D] - cmc$, where the critical micelle concentration, cmc, is the concentration of monomeric surfactant.[9]

The transfer of Cl^- from water to micelles is written in terms of[2c,e,3]

$$K'_{Cl} = [Cl_M^-]/[Cl_W^-]([D_n] - [Cl_M^-]) \quad (3)$$

Equations 1–3 are combined and the computer simulations of the rate data are shown in Figures 1–7, and the rate and equilibrium parameters are in Table I. The second-order rate constants, k_2^m (M^{-1} s^{-1}) are given by

$$k_2^m = 0.14k_M \quad (4)$$

where 0.14 M^{-1} is the assumed volume of the reactive region at the micellar surface.[2c,e,3] If the molar volume of the reactive region at the micellar surface increases with increasing headgroup bulk, values of k_2^m are underestimated by eq 4. Some values of k_M were given earlier[3b] and differ slightly from the present values because better values for K_s and the contribution of reaction with water (Table I) are now available. Values of the cmc are in Table II.

NMR Line Widths. We measured line widths of ^{35}Cl in aqueous solutions with 20 vol % D_2O with variable [NaCl] and constant [surfactant] and with variable [surfactant] and constant [NaCl]. The variations of line width, B, can be fitted to eqs 3 and 5, as in the preceding paper:[5]

$$B = p_W B_W + p_M B_M \quad (5)$$

where p denotes the molar fraction and B the line width at the two sites with the appropriate subscripts W and M. The line width, B_W, has been taken as 8 Hz, but data fits are insensitive to small changes in this value.[5] The lines in Figure 8–13 are calculated

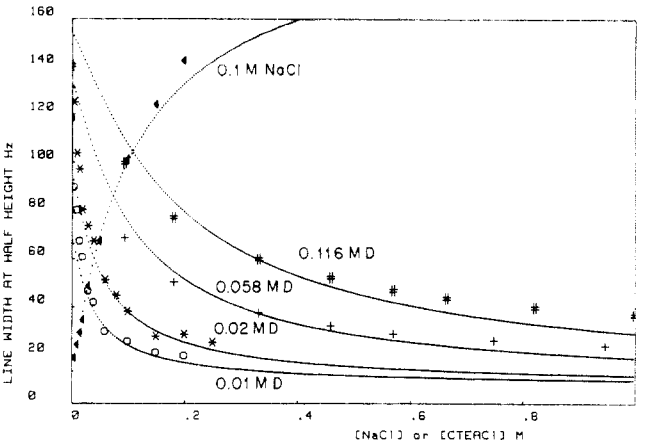

Figure 8. Dependence of ^{35}Cl NMR line width on [NaCl] at indicated [CTEACl] and on [CTEACl] at indicated [NaCl].

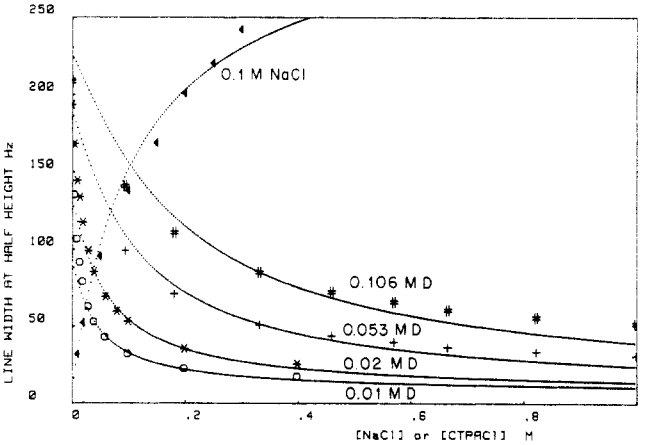

Figure 9. Dependence of ^{35}Cl NMR line width on [NaCl] at indicated [CTPACl] and on [CTPACl] at indicated [NaCl].

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TABLE I: Rates and Equilibrium Constants for Reaction of MeONs and Micellar Interactions with Cl^- [a]

	kinetics					NMR	
	K_s, M^{-1}	K'_{Cl}, M^{-1}	10^5k_M, s^{-1}	$10^5k_2^m$,[c]	$10^5k_M^\circ$,[d] s^{-1}	K'_{Cl}, M^{-1}	B_M, Hz
CTACl[b]	1090	74.2	18.2	2.5	5.8	77.0	204
CTEACl	1000	70.5	25.0	3.5	6.4	68.2	218
CTPACl	1000	50.0	25.1	3.5	9.0	46.6	350
CTBACl	1000	35.0	26.1	3.7	11.0	30.0	472
CTPeACl	213	18.8	90.1	12.6	12.1	19.0[e]	675[e]
CQCl	1000	165.0	32.0	4.5	7.0	150.0	450
CDMHEACl	911	112.0	15.0	2.1	4.4	120.0	300
CTHEACl	700	4300.0	9.1	1.3	3.5	351.0	719

[a] In water at 25.0 °C. [b] Reference 5. [c] For reaction of Cl^- in water $k_W^0 = 1.2 \times 10^{-5}$ s^{-1}.[8] [d] For the spontaneous reaction in water $k_W^\circ = 1.2 \times 10^{-5}$ s^{-1}.[8] [e] Approximate values for 0.05 M CTPeACl based on B = 279.4, 245.6, 197.3, and 195.3 Hz with 0, 0.02, 0.04, and 0.06 M NaCl.

TABLE II. Critical Micellar Concentrations and Aggregation Numbers

	X		
	Cl	$(SO_4)_{1/2}$	N_{agg}[c]
CTAX	1.3 (1.3–1.5)[b]	0.54	75
	0.66 [0.005]		
	0.30 [0.01]		
	0.15 [0.04]		
	0.10 [0.05]		
CTEAX	1.2		
	0.16 [0.05]	0.53	54
	0.071 [0.1]		
CTPAX	0.66		
	0.05 [0.05]	0.39	49
CTBAX	0.52		
	0.18 [0.002]	0.25	32
	0.12 [0.005]		
CTPeAX	0.61	0.27	
	0.046 [0.05]		
COX	1.07		
	0.47 [0.01]	0.48	
	0.19 [0.02]		
CDMHEAX	1.33		
	0.12 [0.05]	0.42	67
CTHEAX	1.02		
	0.093 [0.05]	0.38	65

[a] In water at 25 °C in mM. Quantities in squared brackets are concentrations of added NaCl, M. [b] Reference 12. [c] Aggregation numbers of the chlorides measured by static fluorescence quenching without added salt.[13]

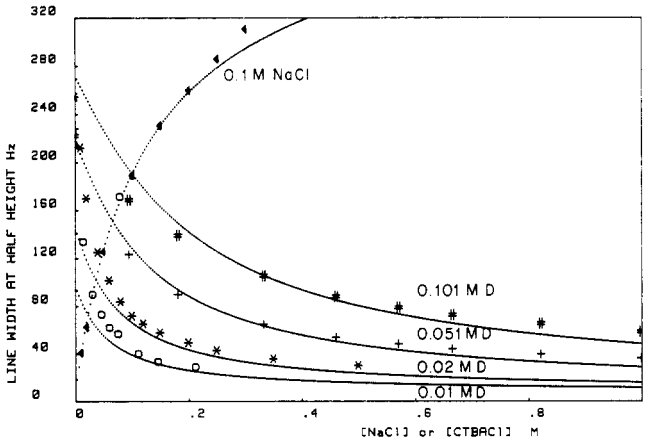

Figure 10. Dependence of ^{35}Cl NMR line width on [NaCl] at indicated [CTBACl] and on [CTBACl] at indicated [NaCl].

by using eq 5 and a distribution of Cl^- calculated with eq 3. The points are experimental. The low solubility of CTPeACl decreased the accuracy of the data for this surfactant (Table I).

Discussion

Micellar Binding of Cl^-. The Langmuir parameters, K'_{Cl}, obtained kinetically are similar to those obtained from the fit of ^{35}Cl NMR line widths, except for CTHEACl (Table I). These

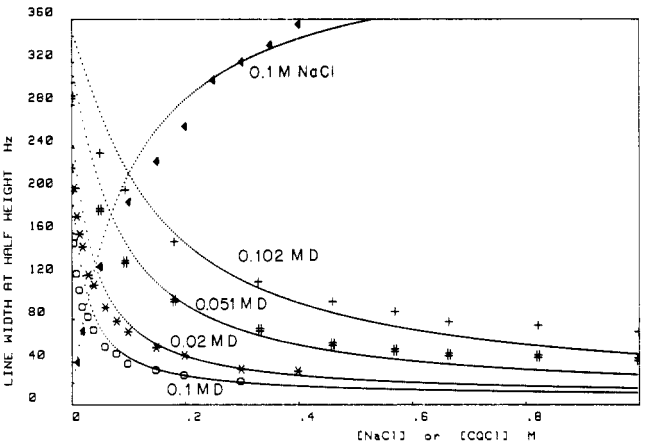

Figure 11. Dependence of ^{35}Cl NMR line width on [NaCl] at indicated [CQCl] and on [CQCl] at indicated [NaCl].

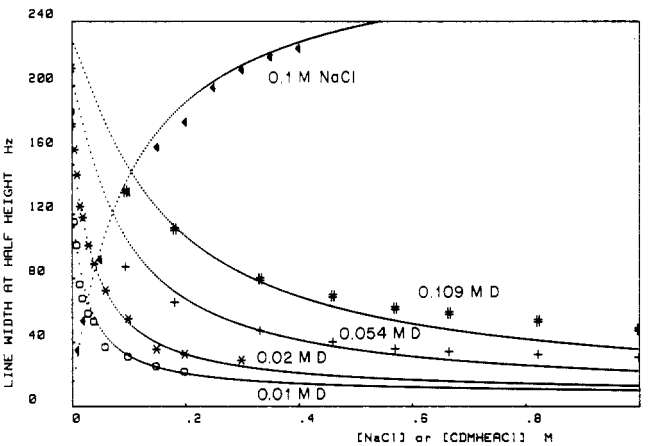

Figure 12. Dependence of ^{35}Cl NMR line width on [NaCl] at indicated [CDMHEACl] and on [CDMHEACl] at indicated [NaCl].

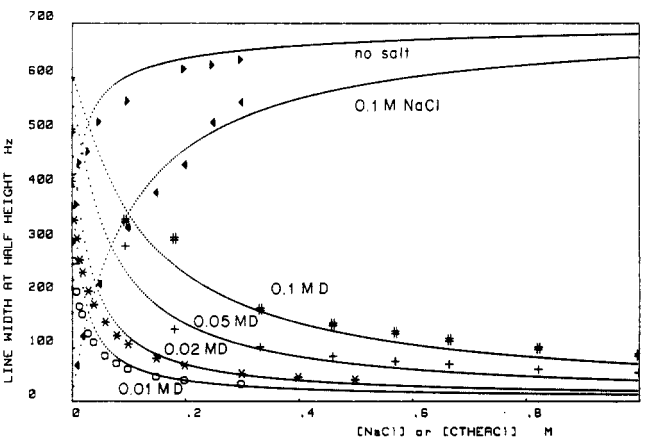

Figure 13. Dependence of ^{35}Cl NMR line width on [NaCl] at indicated [CTHEACl] and on [CTHEACl] at indicated [NaCl].

results are similar to those for alkyltrimethylammonium chlorides[5] but completely different from those for binding of Br^- to cetyltrialkylammonium bromides.[3] Values of K'_{Br} obtained kinetically for reaction of Br^- with MeONs are larger than those from NMR line widths. Bromide ions have a strong specific interaction with cationic micelles, while for Cl^- specific interactions are less important.[2,8b] Cetyl(trihydroxyethyl)ammonium chloride is an exception to the above generalization because its OH groups may specifically solvate Cl^- by hydrogen bonding and displace water molecules, so that it behaves more like a bromide ion surfactant. The hydroxyethyl groups are not involved nucleophilically (Experimental Section).

The two-site model of micellar effects on rates of ionic reactions (e.g., eq 1-3) is an oversimplification because the decrease of counterion concentration from the micellar surface into bulk water has an exponential form,[7,8b,10] whereas the pseudophase ion-exchange model in its simplest forms treats this distribution as a step function.[2b-e] Chemical reactivity senses ions adjacent to micellar-bound substrate and NMR those ions whose hydration shell is perturbed by the headgroup.[11] If a counterion interacts strongly and specifically with micelles, reactivity will probably sense a higher concentration of counterion in the immediate vicinity of the micellar surface than does NMR.[3] The concentration sensed by NMR is smaller because electrostatic interaction between counterions and headgroups attracts ions without the necessity of their intercalating the micellar surface, and these ions can be chemically effective. This situation is seen when Br^- is the counterion,[3] and with Cl^- specifically solvated by OH in the headgroup of THEACl (Table I). In these cases kinetically derived values of K' are larger than those from NMR line widths. Coulombic interactions are relatively long range, so more hydrated, less polarizable, ions, e.g., Cl^-, should be distributed in shells at the micellar surface and will be sensed to similar extents kinetically and by NMR line width. Specific solvation of Cl^- by the hydroxyethyl groups of THEACl increases its effective concentration as regards chemical reactivity without increasing its concentration adjacent to the quaternary ammonium center. Therefore with this surfactant, kinetic and NMR values of K'_{Cl} differ. This conclusion depends upon the location of micellar-bound substrate but NMR chemical shifts suggest that MeONs, like most polar solutes,[2] does not locate deeply in the micelle.[3]

Increase of headgroup size from methyl to pentyl decreases K'_{Cl}, i.e., increases ionic dissociation (Table I). This result agrees with earlier evidence that large alkyl residues at headgroups are "folded back" at the micellar surface, reducing the space between headgroups available for counterions.[3] If there is no "folding back", as with CQCl, or if the residues are hydrophilic, as with CTHEACl or CDMHEACl, values of K'_{Cl} are relatively large (Table I). Our present value of K'_{Cl} for CTACl is lower than that estimated earlier for reaction with little added Cl^-.[8a] Fits of rate–surfactant profiles are not very sensitive to the value of K'_{Cl} under these conditions. Increase of headgroup size reduces micellar size and aggregation number[6] (Table I). This decrease also reduces the space available between headgroups for counterions and increases dissociation of Cl^-.

Substrate Binding Constants. Binding constants of MeONs are unaffected by variations in the headgroup except for CTPeACl (Table I). The three pentyl groups probably occupy so much space at the surface that interaction of the π-system with the headgroups is reduced, as is K_s.

Counterion Solvation and Reactivity. Perturbation of the hydration of Cl^- is seen from the increased line width of micellar-bound Cl^-. Increasing headgroup bulk should affect Cl^- located between the cationic head groups and probably ions in a wider area near to the surface, due to changes in the water structure in that region.[11] A decrease in the symmetry of hydration, as sensed by NMR, involves a decrease in overall hydration and an increase in reactivity, as shown by values of k_M (Table I). This effect is shown not only by bulky groups such as pentyl that "fold back" but also by the quinuclidinyl group, which cannot "fold back" but changes the environment close to the micellar surface. The decrease of ion binding with increase of headgroup bulk is similar in some respects to the widely observed increase in fractional ionization, α, on addition of relatively hydrophobic solutes that associate with micelles.[2c] However, K'_{Cl} is relatively large for CQCl (Table I) because the bulky head group does not "fold back" and exclude Cl^-, although it decreases its hydration and k_M and B_M are larger than for the trialkylammonium micelles.

Hydroxyethyl groups have a special effect. They decrease reactivity of Cl^- by strongly and specifically solvating ions close to the surface, but they also increase the NMR line width (Table I). This behavior is understandable if we accept that interaction of Cl^- with one to three OH groups will give unsymmetrical ionic solvation.

Water reactivity at the micellar surface, as given by values of k_M° (Table I) qualitatively parallels reactivity of Cl^-, because both depend upon perturbation of the water lattice, especially in the region adjacent to relatively hydrophobic micellar head groups. Reactivity of OH^- at the micellar surface also parallels reactivity of Cl^- and increases with increasing bulk of the headgroup.[14]

Experimental Section

Materials. Preparation and purification of surfactants followed standard methods as described,[3-5] except that CTBACl and CTPeACl were prepared from the bromides[3] by ion exchange in EtOH. The chloride surfactants are generally very water soluble, and except for CTPeACl, solubilities increase with bulk of the alkyl headgroups, probably due to decreased interaction of Cl^- with cationic headgroups in the crystal.[3] Solubilities in such organic solvents as $CHCl_3$, C_6H_6, and C_6H_{14} also increase considerably in going from the trimethyl to the tripentyl derivative, but this increase of size of the headgroup reduces water solubility in solutions of the surfactants in organic solvents. Solubilities in water are qualitatively similar for CQCl and CTACl. Cetyltrihexylammonium chloride is a liquid at room temperature and is very soluble in organic solvents but not in water. It apparently has no surfactant properties, probably because the hexyl groups are too large to be accommodated in the space between cationic centers without eliminating all the counterions and disrupting the micelle.

Although CTBACl is very soluble in water, there is a lower consolute temperature on addition of ca. 0.5 M NaCl, and the solubility of CTPeACl is sharply decreased by NaCl. The trihydroxy derivative, THEACl, is paradoxically not very soluble in water, probably because of interactions between OH groups and Cl^- in the crystal. It is also not very soluble in aprotic organic solvents, but this solubility increases very considerably on addition of small amounts of water. It is the most effective of the surfactants in solubilizing water in organic solvents.

Kinetics. Reactions were followed spectrophotometrically at 326 nm, and MeONs was added in 20 μL of MeCN to 3 mL of reaction solution at 25 °C so that [MeONs] = 10^{-4} M.[3,5] The rate constants for the spontaneous reactions in cetyltrialkylammonium sulfate and $CQ(SO_4)_{1/2}$ are from ref 14. Reaction of MeONs in CTHEACl was also followed by ^1H NMR spectroscpy (500 MHz) by monitoring formation of MeCl at 2.839 ppm and free naphthalene-2-sulfonic acid at 7.241 ppm (aromatic CH) with 0.5 M CTHEACl and 0.1 M MeONs in D_2O at 25 °C. The rate constants for formation of MeCl (11.7×10^{-5} s^{-1}) and naphthalene-2-sulfonic acid (10.9×10^{-5} s^{-1}) are similar to the limiting value for the reaction followed spectrophotometrically (12.6×10^{-5} s^{-1}, at [CTHEACl] = 0.08 M). No intermediates were observed. Measurements were made by using a GE-500

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spectrometer and the standard pulse sequence "Kinet" for acquisition of kinetic data. Rate constants were calculated by integration of the signals and exponential fitting with the software of the GE-500 instrument.

^{35}Cl line width and cmc measurements were performed as described.[5]

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Lipid and Lipid–Protein Monolayers Spread from a Vesicle Suspension: A Microfluorescence Film Balance Study

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The process of the formation of a monolayer from a vesicle suspension for the two lecithins DMPC and DPPC, for the phosphatidic acid DMPA, and for a synthetic proteolipid was investigated. Exchange kinetics between vesicles and a monolayer as well as equilibrium spreading pressure of the above substances were determined for different temperatures. The resulting monolayers were separated from the vesicle phase and characterized by film balance microfluorescence techniques. Pressure–area diagrams were taken. Simultaneously the lateral distribution of a fluorescent probe that was a minor constituent of the monolayer was recorded. The data of the pure lipid films were compared with those obtained by the equivalent monolayers spread from an organic solution. The vesicle-spread monolayers were transferred onto solid supports, and the texture and the height distribution of the monolayers were determined. In the case of the lipid–protein monolayer a fluorescence binding assay was carried out after the spreading and transfer procedure. It is shown (1) that the vesicle-spread monolayers are separable from the vesicles, (2) that within the framework of our study their properties do not differ from the equivalent monolayers that were spread from an organic solution, (3) that their composition is the same as that of the vesicles, and (4) that the technique employed here enables one to transfer certain membrane-bound proteins from the vesicle phase via the air–water interface onto solid supports retaining their functionality. Furthermore, a model for the lipid exchange between the vesicles and the monolayer is suggested.

Introduction

Monomolecular lipid films at the air–water interface have been studied extensively in recent years. The interest in these films has on the one hand been stimulated by the relatively easy access to the physical and chemical processes in a two-dimensional system that they offer.[1,2] On the other hand, such monolayers are relevant model systems of biological membranes. The possibility of a monolayer transfer onto a solid support makes them of interest from the point of view of novel materials.[3] These systems have also been demonstrated to be useful in cell surface modeling especially in studies of molecular recognition at cell surfaces.[4] For certain types of problems the use of an artificial membrane system on a solid support offers significant advantages compared to suspended bilayers. Surface sensitive measuring techniques may then be employed. These techniques enable one to distinguish extremely sensitively between events close to the surface and those occurring in the bulk phase.[5] For example, if one of the membranes in a cell–cell interaction can be mimicked by a supported membrane, these techniques offer insight into just the region where the interaction takes place. By means of this the signal to noise ratio of the measurement might be drastically increased.[6]

Monolayers of pure or mixed lipids are spread in general from an organic solution allowing the solvent to evaporate at the air–water interface. This spreading procedure is, however, the main obstacle in the application of the film balance technique to biological systems. Most molecules with biological functions like proteins denature under those conditions. In order to incorporate proteins into such a monomolecular film, other ways of spreading have to be employed. Verger and Pattus[7] formed monolayers by adding dropwise a vesicle suspension onto a vertical glass rod that had its lower end in contact with an aqueous phase. Heckl et al.[33] dropped small amounts of protein in a detergent solution onto a lipid monolayer. Schindler and co-workers investigated the self-assembly of a lipid monolayer at the air–water interface of a vesicle suspension.[8] This latter spontaneous monolayer formation as well as the glass rod method has been used to reconstitute functional channel proteins in black lipid membranes (BLM).[9,10] Since most of the studied molecules were transmembrane proteins, it remained unclear if indeed only a monomolecular film was formed or if a densely packed bilayer structure close to the surface would facilitate the environment for the protein to stay intact. In a more recent work of Kolomytkin[11] it has been shown by measuring current–voltage characteristics that the ion channel amphotericin B can be reconstituted in a black membrane. The author furthermore showed indirectly that this BLM was greatly reduced of attached or partly fused liposomes if the two monolayers obtained according to Schindler's method had to pass a wet bridge of etched glass before the bilayer was formed.

With the motivation to create supported planar lipid bilayers containing intact proteins, we have investigated the monolayer formation from an aqueous suspension of pure lipid vesicles and such vesicles containing the Fab' fragment of an antibody cova-

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