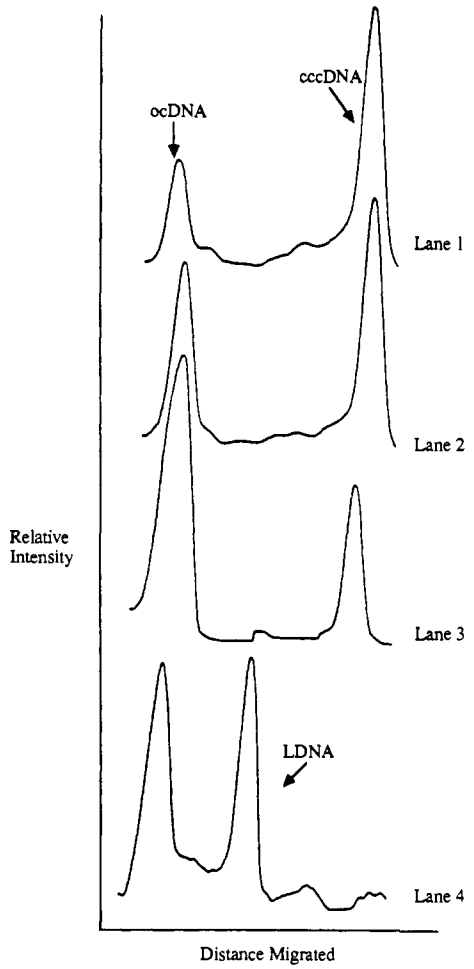

Figure 1. Cleavage of pBR 322 by **1**. All reactions were carried out in 89 mM trizma, 89 mM boric acid buffer (pH = 8.3) in 13 μL total volume at 37 °C in the dark. The concentration of pBR322 was constant at 100 μM in base pairs: lane 1, pBR322 alone, 6 h; lane 2, **1** (7 μM), 6 h; lane 3, **1** (7 μM) and $Cu(OAc)_2$ (48 μM), 6 h; lane 4, **1** (7 μM); $Cu(OAc)_2$ (48 μM), and DTT (1 mM), 3 h. After separation of the DNA by agarose gel electrophoresis, the ethidium bromide stained gel was photographed with a Polaroid MP-4 Land camera equipped with Tiffen (23A) filter and Polaroid Type 665 positive/negative film. The DNA bands were quantitated by densitometric analysis of the negative using a Biorad 620 video densitiometer.

taining hydrogen peroxide (10 μM) and cupric acetate (48 μM) or TMPyP (7 μM), cupric acetate (48 μM) and DTT (1 mM) did not promote DNA cleavage beyond what was observed in the controls, results which confirm the requirement for the aminomethylpyridyl group on **1**. Substantial inhibition (80%) by ethidium bromide (35 μM, 5 equiv based on **1**), a known intercalator, suggests that intercalation of the porphyrin is the binding mode most favorable for the reaction of Cu(II)-**1** with DNA. The inability of the copper complex of 2-(aminomethyl)pyridine to cleave DNA demonstrates that the porphyrin must direct the chemistry of the attached chelator.

With regard to the nature of the cleavage chemistry, the Cu(II)-**1** system may be compared to other known Cu(II) dependent cleaving agents such as bleomycin[15] and 1,10-phenanthroline.[16] Complexed with 1,10-phenanthroline, chelated Cu(I) (generated in situ) is believed to react with hydrogen peroxide (produced via Cu(I) reduction of dioxygen) to form reactive copper species which may then cause site-selective DNA cleavage. The involvement of hydrogen peroxide manifested in the Cu(II)-**1** system suggests a similar redox mechanism.[17,18] Preliminary sequencing studies have indicated that, like copper-bleomycin,[15b] Cu(I)-**1** produced DNA cleavage at discrete sites.[15c] Accordingly, the reactive species responsible for DNA strand scission by **1** is suggested to be a copper species formed upon reaction of hydrogen peroxide with Cu(I)-**1** rather than hydroxyl radical.

These results demonstrate the first example of a DNA cleaving agent which utilizes a porphyrin solely as a DNA recognizing element. With straightforward synthetic modifications, analogues of **1** have been developed which show intriguing variations in cleavage chemistry depending on the nature of the pendant chelator. Clarification of these observations and the possible application of this class of molecules to cancer chemotherapy are currently under investigation.

Acknowledgment. Support of this research by the National Institutes of Health (GM-36298) is gratefully acknowledged.

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Surfactant Structure and Aggregate Morphology. The Urge for Aggregate Stability

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Surfactant aggregation in water is an intricate process. Above a critical concentration, cooperative association sets in and the Gibbs energy of the system is minimized through a compromise of a variety of often opposing forces.[1] These forces depend both on the molecular architecture of the surfactant and on the peculiar solvent properties of water. The urge for optimum aggregate stability is reflected in the rich variety of possible aggregate morphologies, each with its particular mode of alkyl chain packing and headgroup arrangement.[2-6]

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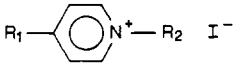
Table I. Aggregation Behavior of the 1-Alkyl-4-(C_{12}-alkyl)pyridinium Iodides **1–9** in Aqueous Solution

compd	aggregate morphology[a]	P^*	cmc × 10^3[b] (mol·kg^{-1})	β[c] (%)	crc × 10^3[d] (mol·kg^{-1})	crc/cmc
1	SM, RM	0.36	2.50	83	45	18
2	SM, RM	0.53	3.93	84	25	6
3	SM, RM	0.58	3.76	80	30	8
4	V	0.91				
5	SM, RM	*e*	2.21	79	37	17
6	SM, RM	*e*	1.91	79	28	15
7	SM, RM	*e*	1.93	76	30	16
8	SM, V	*f*	1.54	79	[~4.5][g]	
9	V	*f*	[~0.8][g]			

[a] SM = spherical micelle, RM = rod-like micelle, V = vesicle. [b] At 30 °C. [c] Counterion binding for SM (from conductivity measurements). [d] At 30 °C in D_2O. For **2** the same crc was found in H_2O at 30 °C. [e] P^* is 0.36 or slightly smaller, compare ref 15. [f] P^* is highly dependent on the headgroup conformation. [g] Critical vesicle concentration.

We report here aggregate morphologies for the 1-alkyl-4-(C_{12}-alkyl)pyridinium iodides **1–9**.[7] We find that minor structural



1, $R_1 = (CH_2)_{11}CH_3$; $R_2 = CH_3$
2, $R_1 = (CH_2)_8C(CH_3)_3$; $R_2 = CH_3$
3, $R_1 = (CH_2)_7CH(CH_2CH_3)_2$; $R_2 = CH_3$
4, $R_1 = CH_2CH(CH_2CH_2CH_2CH_2CH_3)_2$; $R_2 = CH_3$
5, $R_1 = (CH_2)_{11}CH_3$; $R_2 = CH_2CH_3$
6, $R_1 = (CH_2)_{11}CH_3$; $R_2 = (CH_2)_2CH_3$
7, $R_1 = (CH_2)_{11}CH_3$; $R_2 = CH(CH_3)_2$
8, $R_1 = (CH_2)_{11}CH_3$; $R_2 = (CH_2)_3CH_3$
9, $R_1 = (CH_2)_{11}CH_3$; $R_2 = (CH_2)_5CH_3$

perturbations, e.g., branching of the 4-(C_{12}-alkyl) group in the pyridinium ring (**1–4**) and variation of the hydrophobicity of the R_2-alkyl moiety (**1**, **5–9**) leads, depending on the surfactant concentration, to preferential formation of spherical micelles, rod-like micelles, or vesicles. The results are summarized in Table I. Surfactants **1–3** form spherical micelles just above their cmc,[6] but branching near the chain end clearly enhances the propensity of the surfactant molecules to pack into a cylinder at the critical rod concentration (crc).[8] Table I shows the large decrease of crc/cmc upon branching. As discussed by Israelachvili,[9] the effect of branching on the shape of a surfactant molecule can be expressed[10] in a packing parameter $P = V/al$. Herein V is the volume of the hydrocarbon chain, a is the surface area per headgroup in the aggregate, and l is the length of the alkyl chain. Table I lists apparent packing parameters P^*, calculated by using CPK models.[11] For surfactant **4**, $P^* = 0.91$, and now a markedly different behavior is observed. Above a critical concentration, a turbid solution is formed. Either heating to temperatures above 38.5 °C or extended sonication[12] provides a clear suspension, containing bilayer vesicles (diameter 107–120 nm) as evidenced by negative staining (uranyl acetate) and freeze-fracture electron microscopy. Vesicular solutions of **4** could also be directly prepared by the ethanol-injection method.[13] Thus it appears that it is the shape of the surfactant molecule rather than the total hydrophobic volume of the alkyl chain which determines the preferred aggregate morphology.[14]

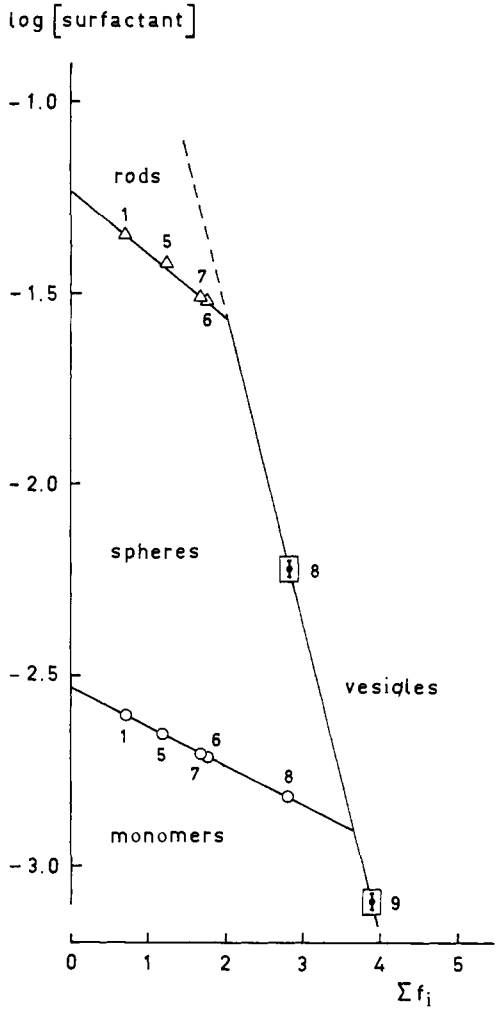


Figure 1. Plot of log[surfactant] vs Σf_i (see text) for aggregates formed from **1** and **5–9** (30 °C): (○), cmc; (△), crc; (□), cvc.

Variation of the 1-alkyl substituent (**1**, **5–9**) also leads to remarkable differences in aggregation behavior. The 4-(*n*-dodecyl)pyridinium iodides **1** and **5–7** form spherical micelles above their cmc's. These cmc's vary only little. Sphere-to-rod transitions[8] are observed at higher concentrations, but the ratio crc/cmc does hardly respond to changes of R_2. Presumably, the 1-alkyl group remains fully exposed to water in the micellar assembly,[15,16] and P^* will remain approximately constant. By contrast, micelles formed from **8** (R_2 = *n*-Bu) are transformed into a lamellar phase at a critical vesicle concentration (cvc). Sonication provides vesicles (diameters between 50 and 160 nm) as shown by electron microscopic techniques. There is a literature precedent that an *n*-butyl group is the shortest headgroup substituent that can fold back into the hydrophobic interior of a surfactant aggregate.[15] Then the packing parameter will increase significantly, leading to preferential bilayer formation. Obviously, the longer 1-*n*-hexyl substituent in **9** also folds back into the core of the assembly, and the surfactant directly forms a lamellar phase which can be easily transformed into vesicles.[17] The results for **1** and **5–9** can be

(7) All surfactants showed satisfactory elemental analyses and were fully characterized by 300 MHz ^{1}H NMR as well as ^{13}C NMR spectral data.
(8) Formation of rod-like micelles was monitored by measuring the abrupt increase of the line width of the ^{1}H NMR resonances at the crc: Olsson, U.; Söderman, O.; Guering, P. *J. Phys. Chem.* **1986**, *90*, 5223.
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(11) P^* values were calculated assuming complete counterion binding and no headgroup hydration. These parameters will be proportional to but not equal to Israelachvili's parameter P.
(12) Compare: Fendler, J. H. *Acc. Chem. Res.* **1980**, *13*, 7.
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conveniently summarized in the diagram depicted in Figure 1. Herein the logarithm of the cmc, crc, and cvc for the different surfactants are plotted versus the sum of the hydrophobic fragmental constants (Σf_i)[18] of the 1-alkyl substituents. Different linear relationships for the three types of aggregates separate concentration ranges for the various aggregate morphologies.

In summary, the present approach demonstrates that the aggregate morphology within a series of 1-methyl-4-(C_{12}-alkyl)-pyridinium iodides of almost equal alkyl chain hydrophobicity is primarily determined by the shape of the surfactant molecule. Shape selectivity also governs the aggregation behavior of 1-alkyl-4-*n*-dodecylpyridinium iodides in which the volume of the core is modified through back bending of a sufficiently long 1-alkyl substituent into the hydrophobic interior of the aggregate. It is our contention that systematic studies of alkyl chain packing will become a major activity in surface chemistry. Further studies along these lines are currently underway in our laboratory.

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(17) No details are as yet known about the exact chain packing in the vesicles formed from **9**. However, interdigitation of the alkyl chains to optimize van der Waals interactions may well be assumed similar to the chain packing in asymmetric phosphatidyl choline bilayer membranes, see: Mattai, J.; Sripada, P. K.; Shipley, G. G. *Biochemistry* **1987**, *26*, 3287. Highly asymmetric di-*n*-alkyl phosphates also form vesicles, see: Wagenaar, A.; Rupert, L. A. M.; Engberts, J. B. F. N.; Hoekstra, D. *J. Org. Chem.*, in press.

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Flavin-Catalyzed Oxidation of Amines and Sulfur Compounds with Hydrogen Peroxide

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Mammalian liver contains liver microsomal FAD-containing monooxygenase (EC 1.14.13.8, FADMO), which oxygenates various amines.[1] The enzymatic oxygenation seems to involve the following catalytic cycle.[2,3a] Oxygenation of enzyme-bound reduced flavin (Enz(FlH_2)) with molecular oxygen gives 4a-hydroperoxyflavin (Enz(4a-FlHOOH)), which undergoes monooxygenation of substrates to give 4a-hydroxyflavin (Enz(4a-FlHOH)). Dehydration of Enz(4a-FlHOH) gives oxidized flavin (Enz(Fl_{ox})) (rate-determining step),[3] which is reduced to Enz-(FlH_2). The mechanism of FADMO has been extensively studied by using 4a-hydroperoxyisoalloxazines (4a-FlOOH), and much understanding has been gained;[4,5] however, the catalytic recycling step is still ambiguous.

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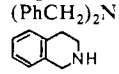
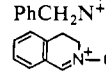
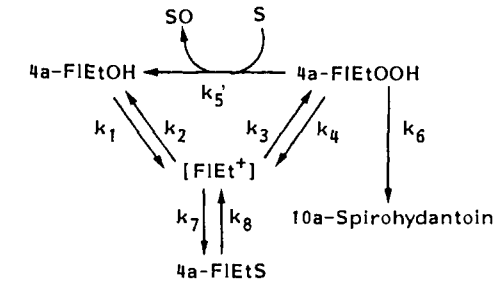
(5) Oxidation of sulfur compounds: (a) Miller, A. E.; Bischoff, J. J.; Bizub, C.; Luminoso, P.; Smiley, S. *J. Am. Chem. Soc.* **1986**, *108*, 7773-7778. (b) Miller, A. *Tetrahedron Lett.* **1982**, *23*, 753-757. (c) Oae, S.; Asada, K; Yoshimura, T. *Tetrahedron Lett.* **1983**, *24*, 1265-1268. (d) Doerge, D. R.; Corbett, M. D. *Mol. Pharmacol.* **1984**, *26*, 348-352.

Table I. Flavin-Catalyzed Oxidation of Amines and Sulfur Compounds[a]

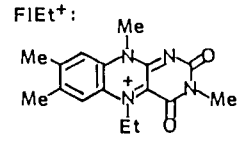
substrate	product[b]	isolated yield, %	turnover number
Bu_2NH	$BuN^+(O^-)$=CHPr	61	12
$(PhCH_2)_2NH$	$PhCH_2N^+(O^-)$=CHPh	40	8
		70	14
$(PhCH_2)_2NOH$	$PhCH_2N^+(O^-)$=CHPh	83[c,e]	8
Bu_2S	$Bu_2S\rightarrow O$	99[c,f]	99
Ph_2S	$Ph_2S\rightarrow O$	96[c]	10
$(PhCH_2)_2S$	$(PhCH_2)_2S\rightarrow O$	97[c,d,e]	19
$(PhCH_2)_2S\rightarrow O$	$(PhCH_2)SO_2$	98[c,d]	10
$(PhCH_2)_2S$	$(PhCH_2)SO_2$	96[d]	19

[a] A mixture of substrate (1 mmol), $FlEt^+ClO_4^-$ (0.1 mmol), and H_2O_2 (2 mmol) in methanol was allowed to react at room temperature under argon. [b] Satisfactory IR, NMR, mass spectral data, and analyses have been obtained. [c] H_2O_2 (1 mmol). [d] CH_2Cl_2. [e] $FlEt^+ClO_4^-$ (5 mol %). [f] $FlEt^+ClO_4^-$ (1 mol %).

Scheme I

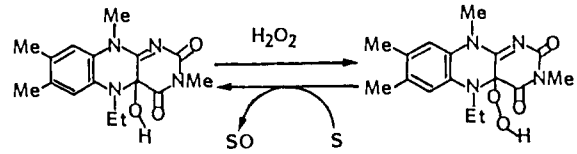


S: Substrate
SO: Oxidized Substrate



We have found that 4a-hydroxy-5-alkylflavins are readily transformed into 4a-hydroperoxyflavins upon treatment with hydrogen peroxide. This result leads to the finding of the novel catalytic oxidation which may correspond to FADMO.

The treatment of 4a-FlEtOH with 30% aqueous hydrogen peroxide (10 equiv) in methanol at room temperature under argon gave 4a-FlEtOOH in 82% isolated yield.[6] Considering this facile formation of 4a-FlEtOOH, 4a-FlEtOH-catalyzed oxidation of substrates with hydrogen peroxide should occur. Indeed, typically, the reaction of dibutylamine (**1**) with aqueous H_2O_2 in methanol



4a-FlEtOH 4a-FlEtOOH

in the presence of 10 mol % of 4a-FlEtOH gave *N*-butylidenebutylamine *N*-oxide (**2**) in 48% isolated yield (turnover number 10). The catalyst is not limited to 4a-FlEtOH, and flavins such as 4a-FlEtOOH,[7] $FlEt^+ClO_4^-$,[8] 5-ethyl-1,5-dihydro-3-methyllumiflavin (FlEtH),[8] FMNHEt,[9] and FMNHMe[9] can be used as an active catalyst, although the flavins which have no substituent at the 5(N)-position such as 3-methyllumiflavin,[10] riboflavin, and

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