Tetrahedron Letters,Vol.30,No.9,pp 1051-1054, 1989
Printed in Great Britain

0040-4039/89 $3.00 + .00
Pergamon Press plc

KINETIC DEMONSTRATION OF PREMICELLAR AGGREGATION. THE *ALKALINE HYDROLYSIS OF N-HEXADECYL-4-*CYANOPYRIDINIUM BROMIDE

N. Hioka*, M.J. Politi** and H. Chaimovich**

*Deptº de Química, Universidade Estadual de Maringá, PR, Brasil. **Deptº de Bioquímica, Instituto de Química, Universidade de São Paulo, C.P. 20780, São Paulo, SP, Brasil.

Abstract: The critical micelle concentration (CMC) of N-hexadecyl-4-cyanopyridinium (HCP) bromide, determined by conductivity or fluorescence quenching, was 7-8 * 10^{-4} M. The observed rate constants for the hydrolysis of HCP below the CMC increased with the concentration of this amphiphile from 2 * 10^{-6} M up to the CMC. The product ratio (pyridone/amide) also varied with HCP concentration. The analysis of both concentration-rate profiles and product composition demonstrated that HCP undergoes premicellar aggregation at concentrations one hundred fold lower than its CMC.

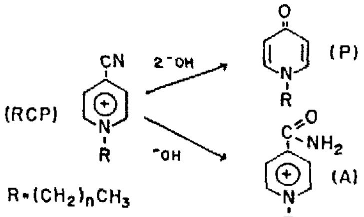
Micelle formation has been generally analyzed using two state models in which monomers and micelles constitute the two relevant states (1,2). However, there is evidence for the existence of premicellar aggregates (3,4). The demonstration of premicellar (self) aggregation is important not only for the theory of micelle formation but also because submicellar aggregates may have relevant chemical properties. Here we show that the observed rate constants for the alkaline hydrolysis of N-hexadecyl-4-cyanopyridinium ions (HCP) vary with concentration below the critical micelle concentration (CMC) for this detergent (HCP). The CMC of HCP obtained by usual methods is shown in Table I.

TABLE I - The CMC of HCP at 30.0 °C.

CMC (M*10^4)	Method	KBr (M*10^2)
8.1	Conductivity	----
7.3	Fluorescence	----
1.9	Fluorescence	0.72
1.6	Fluorescence	2.20

a-Conductimetric CMC determinations were obtained in a Digimed (Brazil) conductivimeter.
b-Fluorimetric determinations were performed in a Perkin-Elmer LS-5 fluorimeter by measuring the extent of Ru(biPy)$^{2+}$ luminescence quenching by HCP monomers (5)

The alkaline hydrolysis of N-alkyl-4-cyanopyridinium ions (RCP) yields the corresponding N-alkylpyridone (P) and N-alkyl-4-carbamidopyridinium (A) (scheme I) (6).



SCHEME I

In excess (or constant) ^{-}OH the observed pseudo first order rate constants (kobs) for hydrolysis of the RCP's, from the methyl to the dodecyl analogs, are identical and independent of substrate concentration (up to 1mM) (5a, 7,8). However, for HCP kobs increased continuously with [HCP], even with concentrations two orders of magnitude below the CMC suggesting the existence of premicellar aggregation (Fig. 1 A-B). Broken lines in Fig. 1 A-B indicate the CMC under kinetic conditions determined at comparable total ionic strength (Table I, third entry). The existence of a clear CMC is in apparent contradiction with the

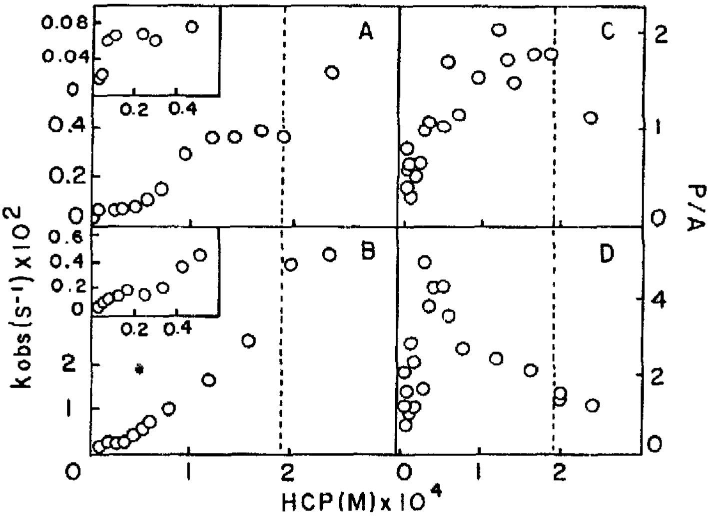


FIG. 1 - Effect of HCP concentration on the rate (A-B) and on P/A ratio (C-D) of alkaline hydrolysis at differents pH's. (A)pH= 9.5, (B)pH= 10.2 (borate buffer, u= 7.2 * 10^{-3}M). The kinetics were followed in a Beckman DU-7 ($t1/2 > 15$ sec) or in a stopped-flow Applied Photophysics-1705 ($t1/2 < 5$ sec), spectrophotometers. The reaction rate was monitored either by the decrease of the absorption of substrate (λ max= 278 nm) or the increase in the absorption of products (λ max= 265 nm). (C)pH= 9.5; (D)pH= 10.2 (buffers as in A & B). The P/A was obtained by the analysis of CN^{-} formed (1:1 with pyridone) or obtained by the molar absortivity of the products measured at 265 nm, E^{A} = 4950 M^{-1} . cm^{-1}, E^{P} = 17400 M^{-1} . cm^{-1} (6,8,9). All runs were made at 30.0 °C.

continuous increase in kobs vs [HCP] below the CMC. This apparent contradiction, must derive from both the difference in the methods and the amplification of the measurement of aggregates afforded by a kinetic method. It is to be expected that both conductivity and fluorescence quenching will be insensitive to minor degrees of (premicellar) aggregation. However if the reaction rate in an aggregate is higher than that of the monomer, the observed reaction rate will reflect this difference, depending on the relative magnitudes of the association and hydrolytic rate constants.

Conclusive evidence that the reaction was also taking place in aggregates was obtained measuring the product ratios (P/A) (Fig. 1 C-D). We have shown that the incorporation of RCP's in micelles (hydrolysis reaction) results in an increase in the P/A ratio (7,8). Even at $1*10^{-5}$ M HCP the P/A ratio is much higher than that obtained in water for the lower homologues, at the same pH. This result may be due to a composite effect of $^{-}$OH concentration at an aggregate and a reaction site of lower effective dielectric constant (8,9). For the sake of clarity we present data for kobs and P/A ratios at selected HCP concentrations (Table II). The variations in these two measurable quantities with [HCP] are evident.

TABLE II - Variation of kobs and P/A ratio for different HCP concentrations at pH= 9.5 and pH = 10.2 (borate buffer), T= 30.0 °C and u= $7.2*10^{-3}$M.

Conc. ($M*10^5$)	pH	kobs ($sec^{-1}*10^4$)	P/A
0.36	9.5	2.0	0.6
5.67	9.5	10.0	1.7
11.83	9.5	34.6	2.0
0.28	10.2	2.7	1.2
5.14	10.2	44.9	4.3
11.86	10.2	163.5	2.4

The P/A ratios were a complex function of [HCP] and were also pH-dependent (*Fig. 1 C-D*). The concentration and pH dependence of the P/A ratios remain to be investigated. However, the important fact to notice is that the P/A ratios were always higher than those obtained, for the lower homologs of RCP, in bulk solution (7).

The literature data concerning premicellar aggregation can be separated in two main groups, one where an added substrate or probe induces an aggregation phenomenon below CMC (10), and one where the detergent self-aggregates (3,4,11). The inducers of premicellar association can also be hydrophobic ionic pair formed between the surfactant and co-ions. As opposed to other systems (12) the only added reagents, aside HCP, in this system were $^{-}$OH and inert salts. It is well known that the binding of $^{-}$OH to micelles and other cationic interfaces is extremely weak (13). Thus, it is extremely unlikely that the premicellar aggregation observed here would have been induced by $^{-}$OH.

In conclusion we have shown that spontaneous premicellar aggregation can be adequately demonstrated using a kinetic method.

Acknowledgments: CAPES-PICD, CNPq, FAPESP & FINEP

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(Received in USA 26 October 1988)