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Bivalent Copper Ion Complex of a Novel Anionic Surfactant Having Functional Imidazole and Hydroxyl Groups as a Remarkably Active Model of Hydrolytic Metalloenzymes

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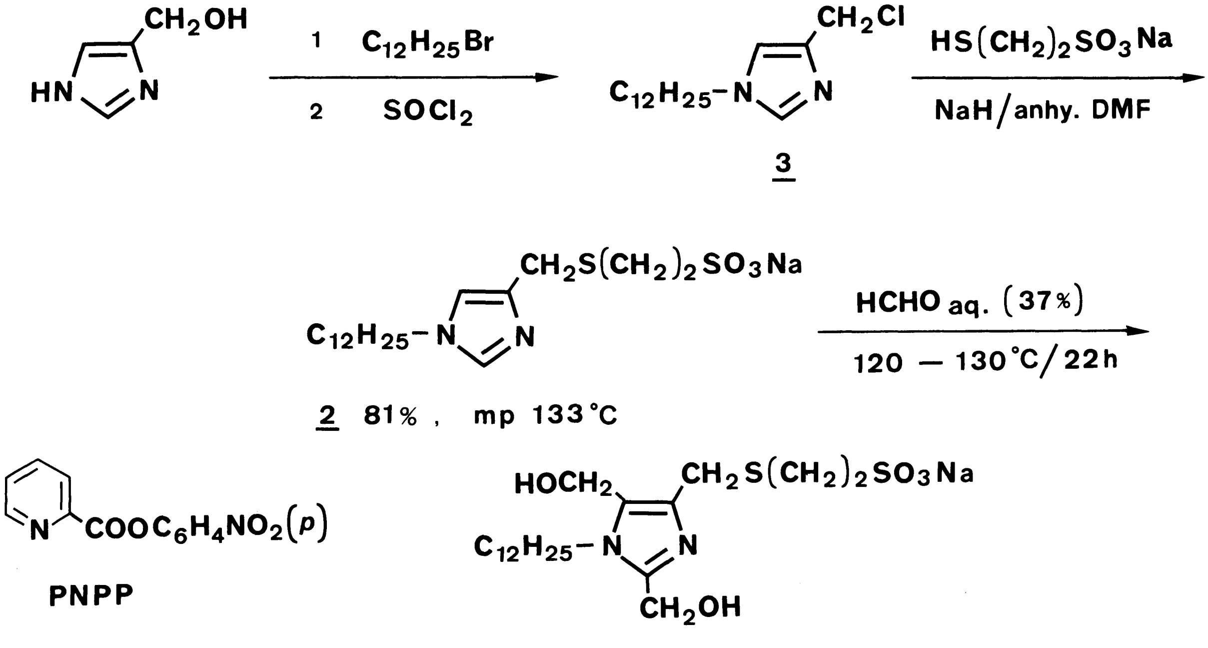
A novel anionic surfactant having functional imidazole and hydroxyl groups, Sodium N-dodecyl-2,5-dihydroxymethyl-4-(2-thiomethylethanesulfonate)imidazole, was synthesized. This surfactant was found to be remarkably active in the acyl transfer reactions of p-nitrophenyl picolinate in the presence of Cu^{2+} ion under micellar conditions.

The micellar catalysis has been extensively studied for the past two decades as the model of enzyme catalysis.1) However, as for the micellar models of hydrolytic metalloenzymes, the studies appear to begin relatively recently. Examples are the metal ion complexes of functionalized imidazoles2) and pyridines 3,4) comicelled with surfactants. Other metal ion complexes of hydroxylamine5) and ethylenediamine6) derivatives were also examined for some ester hydrolysis under micellar conditions. In all of these examples, lipophilic ligands are used to form metal ion complexes and the metal ion sites in the complexes are presumed to act as the polar-head groups of surfactants. However, the lipophilic ligands themselves are not surfactants. We now wish to report a novel anionic surfactant, 1, which exhibits a remarkable activity in the acyl transfer reactions of p-nitrophenyl picolinate (PNPP) in the presence of Cu^{2+} ion under micellar conditions. The surfactant 1 possesses, in addition to a sulfonate polar-head group, an imidazole group for chelation with a metal ion and a hydroxyl group as a nucleophile. This is the first example of a full surfactant model of hydrolytic metalloenzymes, although very primitive.

The surfactant 1 was prepared according to the synthetic pathways shown in scheme 1. The starting 4-hydroxymethylimidazole7) was subjected to the N-alkylation followed by the chlorination to give N-dodecyl-4-chloromethylimidazole 3 according to our previous methods.2) This chloride 3 was reacted with sodium 2-mercaptoethanesulfonate to give sodium N-dodecyl-4-(2-thiomethylethanesulfonate)-imidazole 2.8) Finally, the hydroxymethylation of 2 afforded sodium N-dodecyl-2,5-dihydroxymethyl-4-(2-thiomethylethanesulfonate)imidazole 1.8,9) These 1 and 2 are both typical anionic surfactants.10)

The rates of hydrolysis of substrate PNPP were measured spectrophotometrically by monitoring the release of p-nitrophenol (310 or 400 nm) according to our



3

2 81%, mp 133 °C

PNPP

1 30%, mp 134 °C

Scheme 1.

previous methods.[2] Stopped flow spectrophotomer was used for fast reactions. The results are shown in Figs. 1-3.

Figure 1 shows the plots of the observed pseudo-first-order rate constants (k_{obsd}) as the function of surfactant concentration of 1 or 2. In both cases of 1 and 2, the k_{obsd} values increase with increasing concentration of surfactant in the presence of Cu^{2+} of a fixed concentration to reach a maximum, then decrease gradually. The figure clearly indicates that the maximum k_{obsd} value of 1 is much larger (300 times) than that of 2. It was firmly established in our previous works that the 2-hydroxymethyl group is active for acyl transfer reaction, while the 5-hydroxymethyl group is totally inactive.[2] Therefore, an enhanced activity of 1 as compared to that of 2 is most likely due to the presence of 2-hydroxymethyl group. For more detailed analyses of these curves, it is necessary to obtain more data on the micellization and on the incorporation of Cu^{2+} ion and the substrate into the micellar phase from the bulk water phase.

Figure 2 shows a kinetic version of Job plot[11] which was obtained in order to know the composition of active complex for the reaction. In obtaining this plot, a non-ionic surfactant, Triton X-100,was used as the base surfactant for micellization because of the necessity to change the concentration of 1. The figure indicates a maximum rate at $\gamma = 0.67$ which implies a 2 : 1 complex of 1 and Cu^{2+} as the active complex for the reaction. This γ value is the same as that observed previously for a non-surfactant ligand (N-dodecyl-2-hydroxymethylimidazole).[2a,2c]

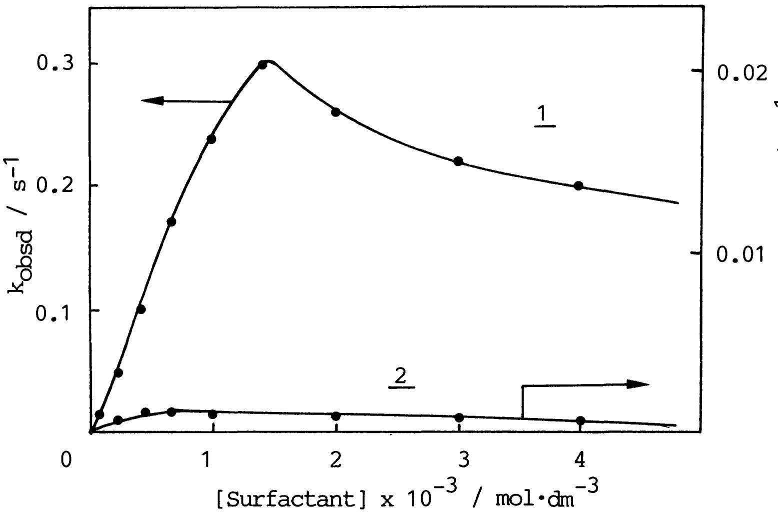


Fig. 1. Dependence of the pseudo-first-order rate constants on the surfactant concentration at 25 °C. $[Cu^{2+}]$ = 2.5 x 10^{-4} mol·dm^{-3}, [PNPP] = 5.0 x 10^{-5} mol·dm^{-3}, an acetate buffer (0.1 mol·dm^{-3} , pH 4.9).

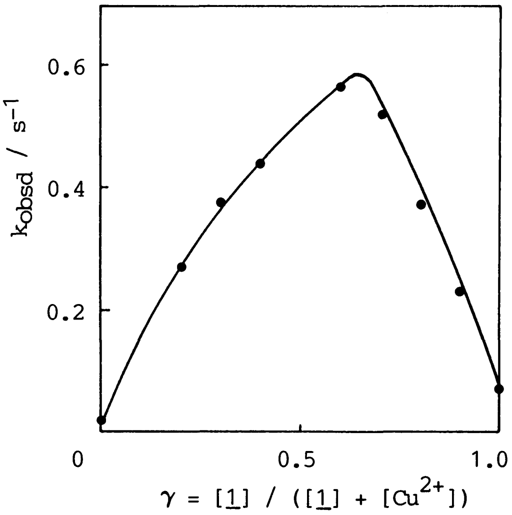


Fig. 2. Job plots for the hydrolysis of PNPP at 25 °C. [Triton X-100] = 2.0 x 10^{-3} mol·dm^{-3}, ([1] + $[Cu^{2+}]$) = 1.0 x 10^{-4} mol·dm^{-3}, [PNPP] = 1.0 x 10^{-5} mol·dm^{-3}, a lutidine-HNO_3 buffer (0.1 mol·dm^{-3}, pH 7.0)

The pH-rate profiles are shown in Fig. 3. The curve A was obtained by using a mixed micelles of Triton X-100 and 1 in a ratio of 20 : 1, while the curves B and C were obtained by using 1 and 2, respectively, without mixing with Triton X-100. The log(k_{obsd})'s in curve A increase linearly with increasing pH with a slope = 1.0 up to pH = 6.2, then level off. This pH-rate profile can be explained as the process of dissociation of Cu^{2+} ion-coordinated 2-hydroxymethyl group of 1 having a pka = 6.2 to an active anion. This pka may be surprisingly small, but it is not unusual when one recalls a well-known fact that the pka of water is reduced from 15.7 to 8.0 on the coordination with Cu^{2+} ion.[12)] The ascending portion of the curves B and C has the same slope of 0.3, much smaller than that of curve A (1.0). This is rather common phenomenon in the dissociation of polymeric acids, since in aggregates any anion formed inhibits the formation of next anion to raise pka of acid groups progressively on dissociation. Thus, in the curve B, the pH 7.8 at which the rates level off seems to indicate an average pka of 2-hydroxymethyl groups of the micellar aggregates of 1.

The above results indicate that a novel surfactant 1 is very active in the liberation of p-nitrophenol from PNPP in the presence of Cu^{2+} ion, but they were obtained by the use of an excess ligand over the substrate, and hence they tell a little about the catalytic efficiency. Burst kinetics were observed in the presence of an excess substrate over the ligand 1. At a lower pH below 7, the reaction practically stops at the liberation of one mole of p-nitrophenol per mole of the ligand, suggesting a rapid acylation of the hydroxyl group of the ligand but a very slow deacylation to regenerate the free hydroxyl group. However, the reaction becomes catalytic by raising the pH. The pH could be raised up to as high as 10.

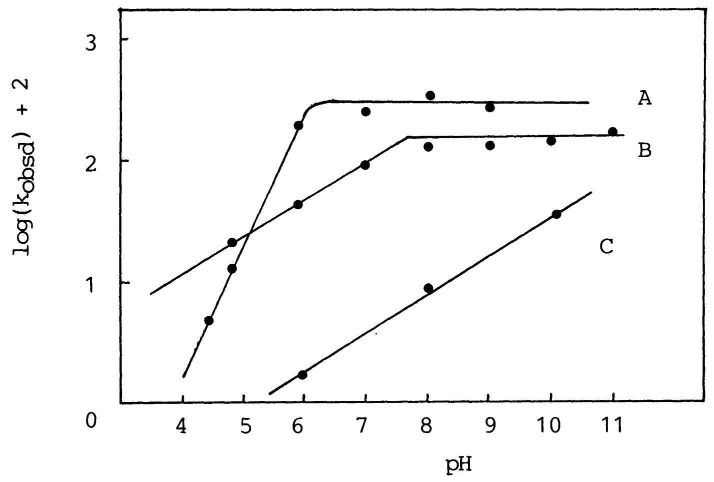


Fig.3. pH-Rate profile for the hydrolysis of PNPP at 25°C. $[Cu^{2+}] = 2.5 \times 10^{-4}$ mol·dm^{-3}, [PNPP] = 5.0×10^{-5} mol·dm^{-3}: A, [Triton X-100] = 1.0×10^{-2} mol·dm^{-3}, [1] = 5.0×10^{-4} mol·dm^{-3} ; B, [1] = 2.5×10^{-3} mol·dm^{-3} ; C, [2] = 2.5×10^{-3} mol·dm^{-3}

Further works are in progress to delineate a more detailed mechanism of this micellar system of 1. This work was partly supported by Grant-in-Aid from the Ministry of Education, Science and Culture in Japan (60470097).

References

1) J. H. Fendler, "Membrane Mimetic Chemistry," Wiley, New York (1982).

2) a) T. Eiki, S. Kawada, K. Matsushima, M. Mori, and W. Tagaki, Chem. Lett., 1980, 997; b) K. Ogino, I. Tomita, T. Machiya, and W. Tagaki, ibid., 1982, 1875; c) W. Tagaki and K. Ogino, Top. Curr. Chem., 128, 143 (1985); d) K. Ogino, N. Kashihara, T. Fujita, T. Ueda, T. Isaka, and W. Tagaki, Chem. Lett., 1987, 1303; e) T. Fujita, H. Minami, K. Ogino, and W. Tagaki, ibid., 1987, 2289.

3) R. Fornasier, D. Milani, P. Scrini, and U. Tonellato, J. Chem. Soc., Perkin Trans. 2, 1986, 233.

4) S. H. Gellman, R. Petter, and R. Breslow, J. Am. Chem. Soc., 108, 2388 (1986).

5) C. D. Gutsche and G. C. Mei, J. Am. Chem. Soc., 107, 7965 (1985).

6) F. M. Mene, L. H. Gan, E. Johnson, and D. H. Durst, J. Am. Chem. Soc., 109, 2800 (1987).

7) J. R. Totter and W. J. Darby, Org. Synth., Coll. Vol. III, 1955, 460.

8) ^{1}H-NMR (CD_3OD, TMS) of 1 supported the structure, Anal. ($C_{20}H_{37}N_2O_5S_2Na$) C, H, N.

9) The hydroxymethylation was carried out in an autoclave. Originally, it was intended to obtain the monohydroxymethylation product at C-2 position. This was achieved occasionally in low yield, but it was more easy to obtain the 2,5-dihydroxymethylation product 1 by prolonged heating.

10) The CMC values and the surface tension at the CMC are : 1, 4.0×10^{-4} mol·dm^{-3} and 28 mN·m^{-1} ; 2, 4.0×10^{-4} mol·dm^{-3} and 32 mN·m^{-1}. The surface tensions were measured by using Shimadzu Surface Tensometer ST-1.

11) A. Martell and S. Chabarek, "Organic Sequestering Agents," Wiley, New York (1985).

12) "Lange's Handbook of Chemistry," 3rd ed, by J. A. Dean, McGraw-Hill, New York (1985).

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