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# ENHANCED REACTIVITIES OF "NAKED" METAL ION AND WATER AFFORDED IN REVERSED MICELLES

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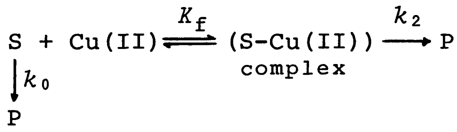
Through the kinetic studies on the hydrolysis of norleucine *p*-nitrophenyl ester in the AOT/$CCl_4$ reversed micelles cosolubilizing aqueous cupric nitrate, we observed evidence of the "naked" copper(II) ion which easily forms a complex with the substrate ester (log $K_f$ = 8 ~ 9) and "free" water molecule acting as an effective nucleophile on the labile complex ($k_{H_2O}$ = 0.2 ~ 0.8 $M^{-1}$ $s^{-1}$).

Ester and peptide hydrolyses as promoted by the metal ions have been a subject of continuing interest over the past decades as a simple model for the metalloenzymes. Nevertheless, efforts in model studies have been spent to determine the actual binding sites of the ligand to the metal ion and to identify the active species in solution.[1] To argue the role of metal ion in metalloenzymes, however, the effect of hydrophobic and very restricted circumstances surrounding the metal ion and substrate should not be ignored. Focusing on the problem, several papers have been recently published; the Mg(II)-ion catalyzed hydrolysis of ATP in the DAP reversed micelles,[2] the ATP hydrolysis as catalyzed by $[Co(II)dien]^{3+}$,[3] and the Mg(II)-ion promoted hydrolysis of phenyl *p*-phosphosulfate in acetonitrile containing a little amount of water.[4] Very recently, through the investigation of solvochromism and thermochromism in the configurational change of cobalt(II) complexes in reversed micelles,[5] the nature and state of "free" water and "naked" (partially dehydrated) metal ions afforded in reversed micelles have been argues.[5] In this communication, we wish to report an interesting feature of the "free" water and "naked" metal ion in the Aerosol OT/$CCl_4$ reversed micelles in conjunction with the hydrolysis of norleucine *p*-nitrophenyl ester (NLPNP).

NLPNP was prepared as described elsewhere.[6] Surfactants, AOT and CTAB, were commercially purchased and purified prior to the use. Reaction rates were followed spectrophotometrically by monitoring the liberation of *p*-nitrophenol upon the hydrolysis (308 nm). The reversed micellar solutions (3.0 ml) cosolubilizing aqueous copper(II) nitrate solution were placed in a thermoregulated cell. Reactions were started by injecting the acetonitrile stock solution of NLPNP ($1.0 \times 10^{-3}$ M) into the cell. Good first-order kinetics were attained in all runs.

NLPNP was spontaneously hydrolyzed in aqueous solution: $k_0 = 1.24 \times 10^{-4}$ $s^{-1}$ at 25 °C, pH 4.88 (acetate buffer), and I = 0.02 M (KCl). As the copper(II) ion was added, the rate was accelerated according to the typical saturation kinetics (Eq. 1).



$$\begin{array}{l} S + Cu(II) \overset{K_f}{\rightleftharpoons} (S\text{-}Cu(II)) \xrightarrow{k_2} P \\ \downarrow k_0 \qquad\qquad\qquad \text{complex} \\ P \end{array} \qquad (1)$$

From the data with different concentrations of copper(II) ion, $K_f$ and $k_2$ were computationally obtained by an iterative calculation[7] (the bottom in Table I). Judging from the previous data obtained for the copper(II) ion catalyzed hydrolysis of ethyl glycinate (log $K_f$ = 3.83 and $k_{obs} = 2.5 \times 10^{-5}\ s^{-1}$ at 25 °C),[8] one may consider that these values are reasonable.

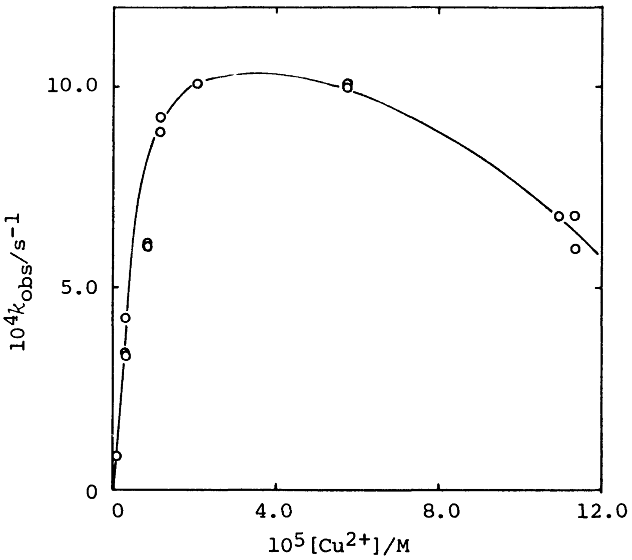


Fig. 1. Effect of the Copper(II) ion concentration on the NLPNP hydrolysis in the 0.10 M AOT/$CCl_4$ reversed micelles at 25 °C. $[H_2O]$=5.5 mM; $[NLPNP]_0$=10 μM

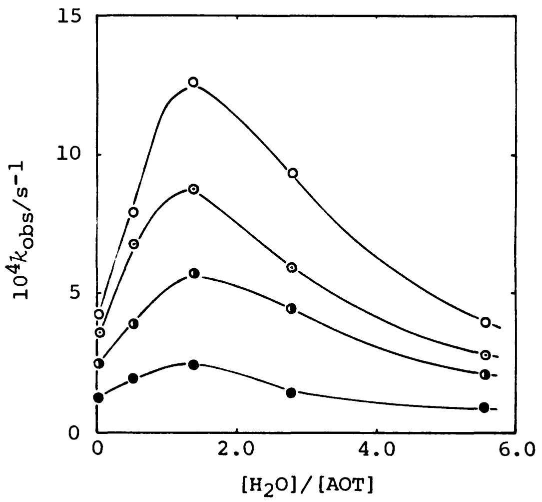


Fig. 2. Effect of water content on the NLPNP hydrolysis as promoted by the copper(II) ion in the 0.10 M AOT/$CCl_4$ reversed micelles at 25 °C. $[NLPNP]_0$=10 μM; $[Cu^{2+}]$= ○, 11.9 μM; ⊙, 7.71 μM; ◑, 5.95 μM; ●, 2.45 μM

On the other hand, in the 0.10 M AOT/$CCl_4$ reversed micelles, the spontaneous hydrolysis was completely inhibited ($k_0 \doteqdot 0$). When the copper(II) ion was added into the system, however, the hydrolysis was significantly accelerated (Fig. 1). Figure 1 suggests that the 1 to 1 complex between the copper(II) ion and substrate is the most reactive species as seen in many amino acid hydrolyses as promoted by metal ions.[1] At given concentrations of water and the metal ion, the rate acceleration was gradually levelled off as increasing the AOT concentration (not shown). Irrespectively of the metal ion concentration, the overall rate constants showed maxima around the point where the concentration ratio, $[H_2O]/[AOT]$, is about 1.0 ~ 1.5 (Fig. 2). It seemed to be closely related with the solvochromism of the cobalt(II) complexes in reversed micelles previously observed,[5,9] where it was interpreted in terms of the *"two step hydration mechanism"* in the reversed micellar sphere.[5,10] During the first hydration stage, the metal ion is partly dehydrated and water is free from the hydrogen bonding with other water molecules.[5,11] Under the condition of $[H_2O]/[AOT] < 1.0 \sim 1.5$, which corresponds to the first hydration stage, the naked metal ion and the substrate will form a strong complex and reactive water will attack on its carbonyl carbon activated by coordination. Upon the completion of first hydration, as water is added the metal ion will be more polyhedrally complexed with water, resulting into the decrease of formation constant to the metal-substrate complex. Water also starts to form the hydrogen bonding each other with a concomitant decrease in the reactivity. The mechanism postulated here is supported by kinetic parameters listed in Table I.

Table I. Kinetic Parameters for the Copper(II) Ion Promoted Hydrolysis of NLPNP in the AOT Reversed Micelles at 25 °C[a)]

| $[H_2O]/[AOT]$ | $\log K_f$ | $k_{H_2O}/M^{-1}\ s^{-1}$ b) |
|---|---|---|
| 0.0495 | ca. 8~9 | 0.0090 |
| 0.545 | 4.0 | 0.15 |
| 1.42 | 3.0 | 0.78 |
| 2.80 | 3.0 | 0.28 |
| 5.55 | 4.3 | 0.0042 |
| In bulk water | 2.6 | 0.00055 |

a) $[AOT]=0.10$ M, $[NLPNP]=1.00\times10^{-5}$ M, and $[Cu^{2+}]=2.45\times10^{-6} - 1.19\times10^{-5}$ M. b) The nucleophilic rate constants of water to the complexed substrates were calculated by dividing $k_2$ (Eq. 1) with the concentration of water added: $k_{H_2O}=k_2/[H_2O]$. For the reaction in the bulk water, $k_2$ was divided by 55 M.

Surprisingly, in the most effective case the nucleophilicity of water to the labile complexes increased by about $10^3$ times in the reversed micelles as compared with that in bulk water. Similarly, the complex formation constant with the naked metal ions was $10^4 \sim 10^5$-fold larger than that with the completely hydrated metal ions. By further addition of water, the nature and state of both metal ion and water will approach to those in bulk water.[5,9,11]

These results obtained here provide two additional important evidence. First, the rate enhancement with increasing water during the first hydration state strongly suggests the attack with internal nucleophiles, not coordinated nucleophiles, on the labile complex.[1] Secondly, judging from the fact that no significant hydrolysis occurred in the CTAB reversed micelles even in the presence of metal ions, one may consider that AOT seems to increase the nucleophilicity of water at least during the first hydration state. In the AOT reversed micelles, the surfactant bearing an anionic head interacts with the hydrogen atom of water, which will result in the increase of nucleophilicity of water. The "naked" metal ions and "free" water would be generated even in polar aprotic solvents.[4] In acetonitrile containing 0.02% water, however, no catalytic effect with the metal ion has been observed. This suggests that the simultaneous incorporation of all three species, the reactive water, naked metal ion, and substrate, in a restricted field is the most important requirement to obtain the effective rate enhancement. The situation afforded by reversed micelles thus resembles the reaction sites of metalloenzymes.

## References and Notes

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10) When the blue aqueous solution of $[Cu(H_2O)_6]^{2+}\cdot 2NO_3^-$ was cosolubilized in the 0.20 M CTAB/$CCl_4$ reversed micelles, it gave an intensely violet solution indicating the formation of $[CuX_4]^{2-}$ species (X = Br) ($\varepsilon_{536}$ = 1160 $M^{-1}$ $cm^{-1}$ and $\varepsilon_{620}$ = 528 $M^{-1}$ $cm^{-1}$). Upon the addition of water the solvochromic effect caused by the configurational change, $[CuX_4]^{2-} + 2H_2O \rightleftharpoons [CuX_4(H_2O)_2]^{2-}$ or $[Cu(H_2O)_6]^{2-}$, was also observed.[5] Though in the anionic AOT micelles the surfactant somewhat interacts with the metal ion,[5] the hydration mechanism in their core should be essentially similar to that in the cationic ones.[11]
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