

Reversed Micelles to Mimic the Active Site of Metalloenzymes

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The structure and reactivity of cobalt(II), nickel(II), and copper(II) halides have been investigated in 0.20 M CTAX (X = Cl, Br)/CHCl₃ reversed micelles. The former two metal ions adopt a tetrahedral configuration at low water concentrations in the micelle. The tetrahedral complexes are converted to octahedral aqua complexes by increasing the water concentration (solvochromism) or by lowering the temperature (thermochromism). Upon reaction with imidazole, the tetrahedral cobalt and nickel halide complexes also undergo a structural transformation into an octahedral configuration with imidazole coordination. At low water concentrations, copper halides form a polynuclear complex bridged by halide ions and these halogen bridges are easily broken upon addition of water or imidazole. The copper complexes produced by reaction with imidazole were deduced to be CuIm₂X₂ and CuIm₄X₂ at intermediate and high ligand concentrations, respectively. It was also found that the cupric ion in reversed micelles is readily reduced to the cuprous ion with 2-mercaptoethanol, and the cuprous ion is oxidized to the cupric ion by reaction with hydrogen peroxide.

Introduction

Reversed micelles, aggregates between surfactants and polar solutes formed in apolar media, provide a specific and restricted reaction field [1, 2]. Depending on the concentrations of surfactant and water, the tightness of surfactant–water interaction, the size of water pool, the microscopic viscosity and polarity of the interior, the reactivity of the water molecule therein can be varied [3]. During the past decade or so, considerable information has been accumulated about the unique feature of reversed micelles in organic reactions [2]. On the other hand, not much

study has been carried out on the nature and behavior of metal complexes in the micelle. We recently reported on the intriguing behavior of transition metal halide complexes in cationic reversed micelles, viz., solvochromism and thermochromism of the cobalt complex [4] and formation of a polynuclear copper complex [5]. Considering the similarity of these complexes to the structure of some of the metalloenzymes, they may mimic the function and structure of the active site of metalloenzymes. In order to elaborate such a metalloenzyme model, the basic properties of the transition metal complexes such as structure and reactivity in reversed micelles have been studied.

Experimental

Materials

Surfactants hexadecyltrimethylammonium chloride (CTACl) and bromide (CTABr) were the same as those used previously [5]. Other reagents were purchased from commercial sources and are of the highest purity available.

Apparatus

Electronic absorption spectra were determined on a Hitachi 200-10 spectrophotometer. The water content in the medium was quantitated with a Kyoto Electronics Karl-Fischer automatic titrator MK-AII.

Results and Discussion

Solvochromism and Thermochromism

Cobalt complexes

As has been reported, cobalt(II) halides undergo solvochromism and thermochromism in cationic reversed micelles [4]. At $R (= [H_2O]/[CTAX])$ values lower than unity, the cobalt(II) ion exhibits

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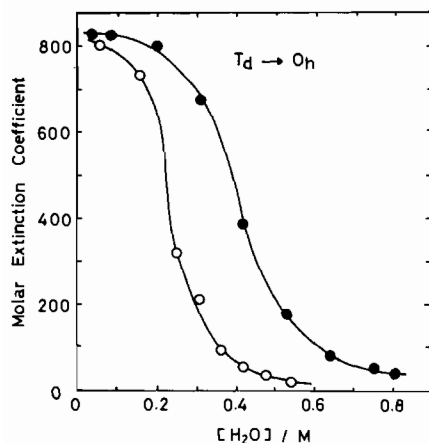
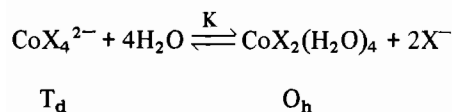


Fig. 1. Transformation of cobalt(II) from a tetrahedral to octahedral configuration (solvochromism) in 0.20 M CTACl (●, 695 nm) or CTABr (○, 719 nm)/CHCl₃ reversed micelles at 25.0 °C.

an absorption band centered at 695 or 719 nm in 0.20 M CTACl or CTABr/CHCl₃ micelles, respectively. These band locations are consistent with the tetrahedral cobalt halogenato complexes, *i.e.* CoCl₄²⁻ and CoBr₄²⁻. As the water concentration in the micelle is increased, the color of the solution fades with a concomitant change in the absorption spectra. A decrease in the absorbance at the respective absorption maxima of the tetrahedral complexes is plotted against water concentration in Fig. 1. This phenomenon is interpreted by a transformation of the tetrahedral complexes to the octahedral aquo complex, CoX₂(H₂O)₄. The data in Fig. 1 reveal that the metal–bromide bond is more readily replaced by water, as may be imagined from the ligand-field strength of the two halides. These data are analyzed by the following scheme:



Scheme 1

The equilibrium constant *K* is defined by eqn. 1, where [H₂O]_f stands for the concentration of water not bound to the metal, and is obtained simply

$$K = \frac{[\text{CoX}_2(\text{H}_2\text{O})_4][\text{X}^-]^2}{[\text{CoX}_4^{2-}][\text{H}_2\text{O}]_f^4} \quad (1)$$

by subtracting the concentration of metal-bound water from the total water concentration (eqn. 2). The combination of eqns. 1 and 2 yields eqn. 3. Since the halide ion is present as the counter ion of sur-

$$[\text{H}_2\text{O}]_f = [\text{H}_2\text{O}]_T - 4[\text{CoX}_2(\text{H}_2\text{O})_4] \quad (2)$$

TABLE I. Standard Free Energy for Conversion of the Tetrahedral Cobalt Halide Complexes to the Octahedral One in Cationic Reversed Micelles.

| Surfactant | $\epsilon_{\text{max}}(\text{T}_d)$ | ln <i>K</i> | ΔG° , kcal mol ⁻¹ |
|--------------------|-------------------------------------|-------------|---|
| CTACl | 830(695 nm) | 0.50 | -0.30 |
| CTABr ^a | 820(719 nm) | 6.23 | -3.69 |

^aTaken from Ref. 4.

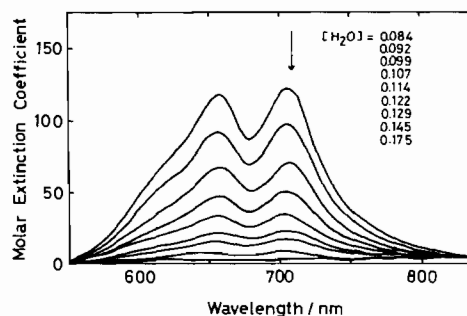


Fig. 2. Transformation of nickel(II) from a tetrahedral to octahedral configuration (solvochromism) in 0.20 M CTACl/CHCl₃ reversed micelles at 25.0 °C. The water concentrations in the micelle are the following from top to bottom: 0.084, 0.092, 0.099, 0.107, 0.114, 0.122, 0.129, 0.145, and 0.175 M.

$$\ln K = \ln \frac{[\text{CoX}_2(\text{H}_2\text{O})_4]}{[\text{CoX}_4^{2-}]} + 2 \ln [\text{X}^-] - 4 \ln [\text{H}_2\text{O}]_f \quad (3)$$

factant in large excess over metal, [X⁻] is set equal to the surfactant in large excess over metal, [X⁻] is set equal to the surfactant concentration (0.20 M). As the extinction coefficient of the octahedral species is negligible in this wavelength region, the observed absorbance is directly proportional to the molar fraction of the tetrahedral species α (eqn. 4), where *M*₀ and ϵ denote the total metal concentration (0.90

$$A = \alpha \cdot \epsilon \cdot M_0 \quad (4)$$

mM) and molar extinction coefficient of the tetrahedral complex at its absorption maximum wavelength. Thus, eqn. 3 is rearranged into eqn. 5, from which *K* is obtained. The results are summarized in Table I.

$$\ln \frac{1 - \alpha}{\alpha} = 4 \ln [\text{H}_2\text{O}]_f + \ln K + 3.219 \quad (5)$$

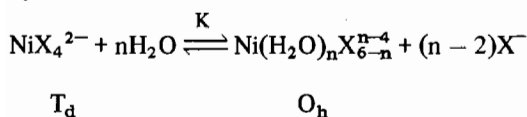
Nickel complexes

In Fig. 2 are shown the spectra of nickel chloride in 0.20 M CTACl/CHCl₃ reversed micelles. At the

TABLE II. Thermodynamic Parameters for the Transformation of NiX_4^{2-} to $\text{Ni}(\text{H}_2\text{O})_6^{2+}$.

| Surfactant | ϵ_{max} (T_d) | $\ln K$ | ΔG° , kcal mol^{-1} | ΔH° , kcal mol^{-1} | ΔS° , e.u. |
|------------|-----------------------------------|---------|--|--|----------------------------|
| CTACl | 323(705 nm) | 8.50 | -5.02 | -26.2 | -71.0 |
| CTABr | 297(745 nm) | 13.16 | -7.78 | -34.4 | -89.3 |

lowest water concentration studied (0.084 M), the metal exhibits absorption maxima at 655 and 705 nm. These absorption bands are characteristic for the tetrahedral nickel complexes. As the water concentration in the micelle is increased, the absorbance of the two bands decreases with a concomitant build-up of a new band at 395 nm, which is assignable to the hydrated octahedral nickel complex, $\text{Ni}(\text{H}_2\text{O})_6^{2+}$ (see below). In the CTABr micelle the metal behaves quite analogously: the original tetrahedral complex, NiBr_4^{2-} , showing absorption maxima at 698, 745, and 1415 nm is converted to the octahedral aqua complex with an increase in the water concentration (data not shown). These phenomena are explicable by the scheme shown below:



Scheme 2

The data were analyzed in a way analogous to that described for the cobalt complexes. The hydration number of the octahedral species (n) was determined as 6 by trial and error calculations. The results obtained by this data processing are summarized in Table II. It is obvious that the equilibrium constants for the formation of the octahedral complex are much larger with nickel than with cobalt, in agreement with the general idea that the former metal assumes an octahedral configuration more readily than the latter.

Nickel halogenato complexes undergo thermochromism in CTAX/ CHCl_3 reversed micelles at an R value of ~ 0.5 . As the temperature is raised from 20 to 60 °C, the absorption of the tetrahedral complexes increases. These phenomena are explained on the same basis as that of the solvochromism (Scheme 2). Since more water is partitioned in the bulk solvent phase with the rise in temperature, the interior core is deprived of water, thus shifting the equilibrium towards the tetrahedral complex. The equilibrium constants determined for both nickel chloride and bromide at different temperatures ranging from 20 to 60 °C (8–10 points) are utilized for evaluating the standard enthalpy and entropy associated with

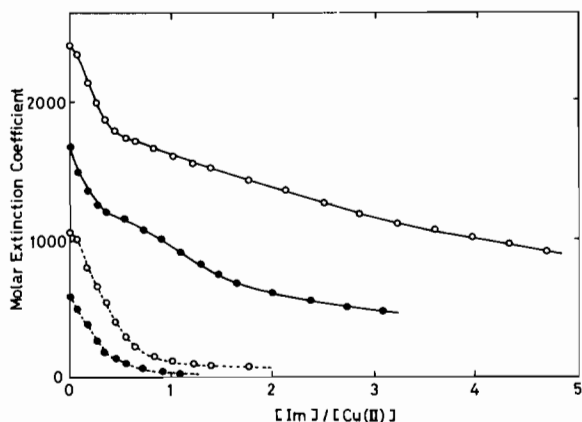


Fig. 3. Decreases in the absorption of the polynuclear copper(II) complex (0.68 mM) upon reaction with imidazole in 0.20 M CTACl/ CHCl_3 reversed micelles containing 0.057 M (○) and 1.02 M (●) water at 25.0 °C. The absorbance was determined at 294 (solid lines) or 408 nm (broken lines).

this configurational change (Table II). The transition of the tetrahedral complex to the octahedral one is characterized by a large negative entropy, while it is a favorable process in terms of enthalpy.

It should be remarked that similar thermochromism of nickel halogenato complexes has been reported in water. For example, nickel ion is converted to NiCl_4^{2-} at high temperature in the presence of a 200-fold excess of tetramethylammonium chloride [6]. The enthalpic change of this transformation is about $-14 \text{ kcal mol}^{-1}$. The tetrahedral nickel complex (NiCl_4^{2-}) is also generated in aqueous media containing a high concentration of MgCl_2 at high temperature ($>125^\circ\text{C}$) [7]. In this case the equilibrium is between NiCl_4^{2-} and NiCl_6^{4-} , and the enthalpic change for this transformation is $-8.5 \text{ kcal mol}^{-1}$. Thus, the structural transformation of nickel complexes in reversed micelles takes place at a lower energy.

Reaction with Imidazole

Copper complexes

Cupric chloride forms a diamagnetic complex of polymeric nature in the cationic CTACl/ CHCl_3

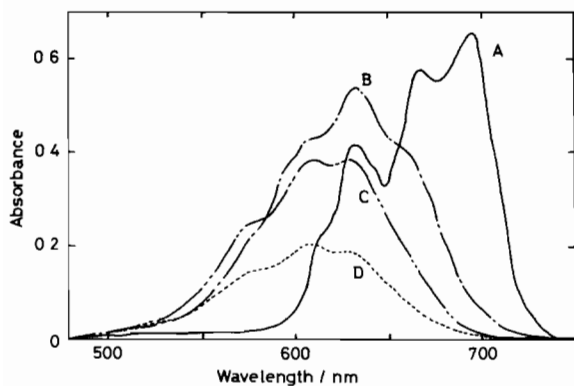


Fig. 4. Reaction of tetrahedral cobalt(II) complex (0.90 mM) in 0.20 M CTACl/0.054 M H₂O/CHCl₃ reversed micelles at 25.0 °C. The molar ratio of imidazole to cobalt is 0(A), 4.2(B), 20(C), and 26(D).

micelles [5]. The complex is characterized by its absorption maximum at 408 nm as well as those at 294 and ~1150 nm. The absorption at these wavelengths decreases sharply upon addition of imidazole (Fig. 3), indicating that the polymeric copper complex bridged by chloride ions is being destroyed by imidazole. The decay curves determined at 294 and 408 nm are not parallel with each other, because the former band is obscured by spectral overlap with that of the resulting copper-imidazole complex. Hence, the absorbance at 408 nm reflects better the fate of the original complex. Extrapolation of the initial linear portion of the decay curve to zero absorbance indicates that 0.65 mol of imidazole per copper is sufficient to destroy the chloride bridge. (The change in the ligand field band of copper is plotted against the molar ratio of imidazole to metal in Fig. 3). As the imidazole concentration is increased, a new absorption band becomes observable at 830 nm with a concomitant loss of absorbance at ~1150 nm. During this change an isosbestic point was obtained at 1065 nm. Along with these spectral changes, the copper ion becomes EPR positive by the addition of imidazole. The metal exhibits a single broad signal at $g\ 2.12 \pm 0.02$ in the 0.20 M CTACl/0.063 M H₂O/CHCl₃ micelle at room temperature. The signal intensity grew linearly with increasing imidazole concentration up to ligand to metal ratio ~4 (data not shown), whereupon a blue crystalline mass precipitated out. By reference to the literature [8], this complex was identified as CuIm₄Cl₂.

In the CTABr/CHCl₃ micelle, the polymeric cupric halogenato complex reacts with imidazole as in the CTACl micelle. However, the complex produced in the presence of 2- to 3-fold excess imidazole over metal shows an absorption maximum at 850 nm, unlike at 830 nm in the CTACl micelle, indicating that the halides are coordinating to the metal in these

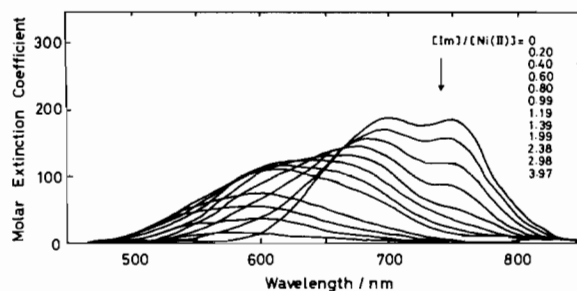


Fig. 5. Reaction of tetrahedral nickel(II) complex (2.03 mM) in 0.020 M CTABr/0.042 M H₂O/CHCl₃ reversed micelles at 25.0 °C. The molar ratio of imidazole to nickel is the following from top to bottom: 0, 0.20, 0.40, 0.60, 0.80, 0.99, 1.19, 1.39, 1.99, 2.38, 2.98 and 3.97.

complexes. These band locations are similar to those of the tetrahedral copper complexes such as Cu(2-MeIm)₂Cl₂ (835 nm) [9] and Cu(1,2-diMe-Im)₂Cl₂ (901 nm) [10]. It is therefore likely that the soluble copper imidazole complexes obtained in the reversed micelle have a composition like CuIm₂X₂. To our knowledge, such a copper imidazole complex has never been observed in solution especially in the presence of water. Furthermore, the absorption spectra of the complexes resemble that of copper-carbonic anhydrase in shape [11], suggesting that they assume a distorted tetrahedral configuration. At a molar ratio of ~3 of imidazole to copper in the CTABr micelle, a blue precipitate starts to form. This complex was found to be CuIm₄Br₂ (see above).

Cobalt complex

The tetrahedral cobalt(II) halogenato complexes, CoX₄²⁻, present predominantly at low water concentrations in cationic reversed micelles, undergo a structural transformation upon addition of imidazole, as revealed by a red-shift of the 695 and 667 nm bands (Fig. 4-B). The absorbance of the complex thus produced decreased with little change in the absorption maxima with a further addition of imidazole (Fig. 4-C and D). Hence, it is evident that at least two transformations are involved over the imidazole concentration range studied. The complex generated by the first transformation is deduced to be CoIm₂X₂ on the basis of the spectral similarity. Judging from the small extinction coefficient, the complex formed after the second transformation is likely to be an octahedral complex like [CoIm_nX_{6-n}]ⁿ⁻⁴ with an n value of greater than 3.

Nickel complex

Like cobalt, the tetrahedral nickel halogenato complexes underwent a spectral shift to the blue upon addition of imidazole (Fig. 5). The complex at high imidazole concentrations possesses a λ_{\max}

at 580 nm and is likely to be an octahedral complex such as NiIm_4X_2 and NiIm_6X_2 . A notable difference from the cobalt complex is that a tetrahedral nickel complex with an imidazole coordination was not observed at intermediate ligand concentrations, presumably because nickel prefers an octahedral configuration to a tetrahedral one more readily than cobalt does.

Oxidation and Reduction of Metal Complexes

Many metalloenzymes perform their function through oxidation and reduction of the central metal ions. In this context, the redox properties of the transition metal complexes described above have been studied. Anhydrous cuprous chloride, solubilized in 0.20 M CTACl/CHCl₃ reversed micelles at $R \sim 1$, does not give appreciable absorption in the visible-ultraviolet region. Addition of small quantities of hydrogen peroxide gave rise to an absorption band at 408 nm, indicating that the cuprous ion has been oxidized to produce the polynuclear cupric chloride complex. Conversely, the polymeric cupric complex thus prepared is readily reduced back to the cuprous ion with a common reducing agent such as 2-mercaptoethanol. The reduction takes place rapidly, but the exact structure of the reduced complex, e.g. coordination of the disulfide to the metal, is not clear at present. At any rate, facile oxidation and reduction of copper in reversed micelles should be useful in constructing a catalytic oxidation reaction system involving the metal. Neither cobalt(II) nor nickel(II) were oxidized or reduced under identical conditions.

Metal Ion Promoted Reactions in Reversed Micelles

These unique structural characteristics of transition metal complexes in the cationic reversed micelles are evident in reactions promoted by metal ions in aqueous media.

The hydrolysis of norleucine p-nitrophenyl ester was accelerated drastically by copper ion in 0.10 M AOT reversed micelles in CCl₄ [12]. The effect of copper was maximal at low water concentration (~ 0.15 M) in the micelle, and a further increase in water concentration diminished the rate of acceleration by the metal sharply. The intramolecular cyclization of the pyridoxal Schiff base of

histidine is also affected by metal ions in reversed micelles [13]. Of interest is the fact that even the magnesium ion was effective in prohibiting the cyclization in the micelle, while it is totally ineffective in non-micellar solutions. All these phenomena of metal ions may be explained by their 'naked' character in reversed micelles, especially at low water concentrations.

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References

- 1 J. H. Fendler and E. J. Fendler, 'Catalysis in Micellar and Macromolecular Systems', Chapter 10, Academic Press, London, 1975.
- 2 J. Sunamoto, 'Solution Behavior of Surfactants - Theoretical and Applied Aspects' (K. L. Mittal and E. J. Fendler, Eds.), Vol. 2, Plenum, New York, 1983.
- 3 J. Sunamoto, T. Hamada, T. Seto and S. Yamamoto, *Bull. Chem. Soc. Jpn.*, **53**, 853 (1980).
- 4 J. Sunamoto and T. Hamada, *Bull. Chem. Soc. Jpn.*, **51**, 3130 (1978).
- 5 J. Sunamoto, H. Kondo, T. Hamada, S. Yamamoto, Y. Matsuda and Y. Murakami, *Inorg. Chem.*, **19**, 3668 (1980).
- 6 M. K. Scarrow and T. R. Griffiths, *Chem. Commun.*, 425 (1967).
- 7 C. A. Angell and D. M. Gruen, *J. Am. Chem. Soc.*, **88**, 5192 (1966).
- 8 W. J. Eilbeck, F. Holmes and A. E. Underhill, *J. Chem. Soc. (A)*, 757 (1967).
- 9 W. J. Eilbeck, F. Holmes, C. E. Taylor and A. E. Underhill, *J. Chem. Soc. (A)*, 128 (1968).
- 10 D. M. L. Goodgame, M. Goodgame and G. W. R. Canham, *J. Chem. Soc. (A)*, 1923 (1971).
- 11 C. A. Angell and D. M. Gren, *J. Phys. Chem.*, **70**, 1601 (1966).
- 12 J. Sunamoto, H. Kondo and K. Akimaru, *Chem. Lett.*, 821 (1978).
- 13 J. Sunamoto, J. Kondo, J. Kikuchi, H. Yoshinaga and S. Takei, *J. Org. Chem.*, **48**, 2423 (1983).