

A STUDY ON AMIDE HYDROLYSIS CATALYZED BY METAL COMPLEXES

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This study is involved to develop new catalysts to decompose plastics, detergents and surfactants containing synthetic peptide bonds. As the first year research, the catalytic hydrolysis of amide bond in copper complex was accomplished. The hydrolysis reaction in aqueous solution was monitored by UV/VIS spectroscopy. As the pH of the solution is increased and the temperature is raised, the reaction rate increases. The reaction rate is observed as the first order kinetic behavior for the copper complex. The metal catalyzed hydrolysis mechanism is proposed via metal-hydroxide in the pH region of 5.5 to 6.3. The results of characterization of the catalytic reaction mechanism can be applied to develop new catalysts for peptide bond degradation in further research.

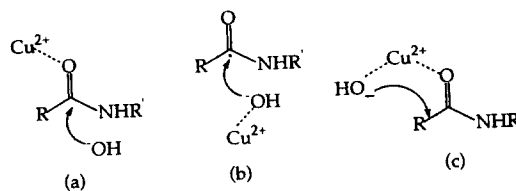
Key words :

1. INTRODUCTION

Nowadays technology has grown up that produces hundreds of substances containing synthetic macromolecules. In parallel with the synthesis of the polymeric substances it is greatly needed to develop catalysts for degradation of these to reduce environmental damage such as earth and water pollution. There are many goods such as detergents, surfactants and plastics which contain polypeptide bonded materials. (Joesten. et al, 1990) The ideal catalysts for degradation must be a mimic of naturally occurring enzymes, which can be applied for synthetic peptides. As a part of research to develop catalysts to decompose peptide bonds, we investigated the catalytic process in copper amide complex for the amide bond decomposition.

The mechanism of peptide bond hydrolysis catalyzed by the zinc-containing peptidases has been intensely investigated for many years. Several different mechanisms have been suggested for the action of carboxypeptidase A. (Schepartz and Breslow, 1987) Most of them have involved an inter-

mediate in which carbonyl oxygen is coordinated to the zinc enzyme since Lipscomb found it in XRD study. (Christianson and Lipscomb, 1989; Groves and Baron, 1989) Numerous amide hydrolytic metalloenzyme models have been focused on coordination of amide carbonyl to the metal to investigate mechanistic role of metal ion. (Buckingham et al., 1974; Chin et al., 1993; Collman, and Kimura, 1967; Curtis and Sargeson, 1984) Generally two or three mechanisms have been known for amide hydrolysis. (Chin, 1991; Chin et al., 1990) The first one is Lewis acid mechanism involving direct substrate activation by metal-carbonyl coordination (a) and another is metal-hydroxide mechanism involving water activation (b). Chin et al. (1990) proposed a combined mechanism (c). Both of the (a) and (c) are involving metal-carbonyl coordinated species.



We have synthesized a new copper (II)-amide complex, $\text{Cu}(\text{dpba})(\text{NO}_3)_2$ ($\text{dpba}=2,2'$ -dipyridylbenzamide) in which the ligand amide carbonyl is not coordinated to the metal by an X-ray crystal structural analysis. (Lee et al., 1996) We tried to investigate a catalytic hydrolysis of amide ligand in copper complex, in which the metal-carbonyl oxygen intramolecularly coordinated complex cannot be involved both in starting complex and in intermediate. In this paper the hydrolysis of the 2,2'-dipyridylbenzamide ligand catalyzed by copper intramolecularly in pH 5.5~6.3 solution is reported.

2. Experimental Section

$\text{Cu}(\text{dpba})(\text{NO}_3)_2$ was prepared from the reaction of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ and dpba in ethanol and recrystallized in $\text{CH}_3\text{CN}^{10}$. The buffer solution was made with CH_3COOH or MES and NaOH or HCl. The amide ligand hydrolysis was accomplished by dissolving the $\text{Cu}(\text{dpba})(\text{NO}_3)_2$ in the buffered solution to produce $\text{Cu}(\text{dpba})(\text{H}_2\text{O})_n^{2+}$ species. The reaction was monitored by UV/VIS spectroscopy on a Varian Cary 5 UV-VIS-NIR spectrophotometer. Good first order plots were obtained which were linear for at least 5 half lives. Absorption at 400 nm was measured as the reaction proceeded. In a typical experiment, copper complex (5.0 mM) was dissolved in pH 5.5 to 6.3 buffered solution ($\text{CH}_3\text{COONa}/\text{CH}_3\text{COOH}$ or MES buffer). The acidity of the solution did not change by more than 0.2 pH unit.

3. Results and Discussion

Fig. 1 shows the electronic spectral change as the reaction proceeds. Spectrum A for copper amide complex is changed to spectrum B. As expected hydrolyzed products are copper amine complex and benzoic acid. We individually synthesized the copper amine complex and measured the electronic spectrum. We obtained the same electronic spectrum as B. Another product, benzoic acid was fully characterized with gas chromatographic me-

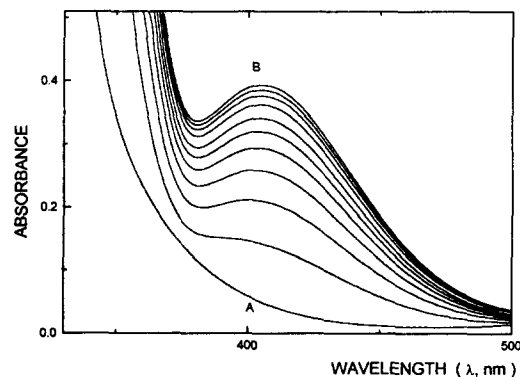


Fig. 1. Electronic spectra of Amide Hydrolysis. The absorbance increases as the reaction proceeds from A to B and time interval is 4 hours (MES buffer, pH 6.3, 23°C).

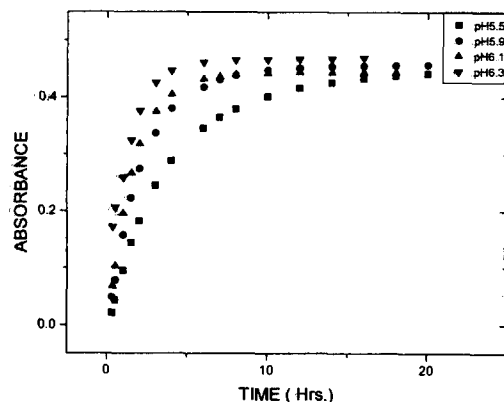


Fig. 2. Absorbance vs. Time curve Observed at 400 nm for Amide Hydrolysis (44.8°C, pH 6.3, $[\text{Cu}(\text{II})]=5 \times 10^{-3}\text{M}$, 0.1 M MES buffer)

thod. With the progress of the reaction, absorption at 400 nm is increased as shown in Fig. 2. Plots of $\ln(A_{\infty}/A_t)$ vs. time show linearity (Fig. 3) and the pseudo first order rate constants observed at 37.5°C, k_{obs} are 5.4×10^{-3} , 4.9×10^{-3} , $2.8 \times 10^{-3} \text{ min}^{-1}$ at pH 6.3, 5.9 and 5.5, respectively. The first order kinetic behavior for metal complex has been observed. Therefore, amide ligand hydrolysis in copper complex is intramolecularly catalyzed by copper.

Fig. 4 is plots of $1/k_{\text{obs}}$ vs. $[\text{H}^+]$ showing that the reaction rate increases as $[\text{H}^+]$ is decreased in

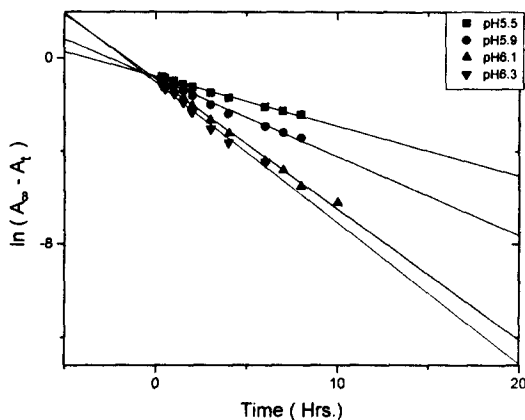


Fig. 3. Plots of $\ln(A_{\infty} - A_t)$ vs. Time for the Amide Hydrolysis (45°C , pH 6.3, $[\text{Cu}(\text{II})] = 5 \times 10^{-3}\text{M}$, 0.1 M MES buffer)

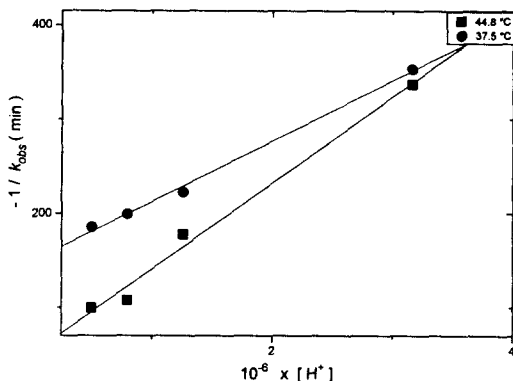
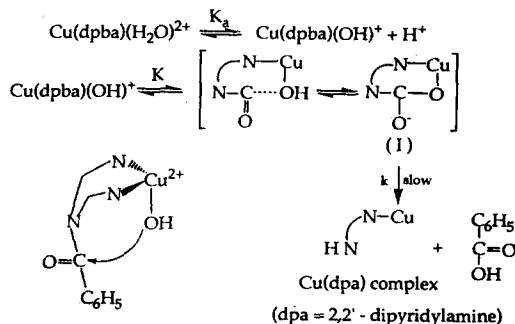


Fig. 4. Plots of $1/k_{\text{obs}}$ vs. $[\text{H}^+]$ ($[\text{Cu}(\text{II})] = 5 \times 10^{-3}\text{M}$, 0.1 M MES buffer)

the range at pH 5.5 to 6.3. We propose the mechanism for this amide ligand hydrolysis through copper-hydroxide (scheme I). Ball and stick model of this copper complex indicates that the copper-bound hydroxide in axial site is in proper position, nearly in contact with acyl carbon of the amide.



Scheme I

In acidic condition of pH 5.5~6.3 water bound $\text{Cu}(\text{dpba})(\text{H}_2\text{O})_n^{2+}$ and hydroxide bound $\text{Cu}(\text{dpba})(\text{H}_2\text{O})_{n-1}(\text{OH})^+$ coexist in equilibrium. A pK_a of water bound complex at 23°C was measured with a value of 6.4 by titration with NaOH. The hydrolysis reaction rate is expressed as follows.

$$\begin{aligned} \text{rate} &= -d[\text{I}]/dt \\ &= k[\text{I}] \\ &= k K [\text{Cu}(\text{dpba})(\text{OH})^+] \\ &= k K K_a [\text{Cu}(\text{dpba})(\text{H}_2\text{O})^{2+}] / [\text{H}^+] \end{aligned}$$

When we rearrange the rate law in terms of total $\text{Cu}(\text{II})$ concentration, $[\text{Cu}(\text{II})]_{\text{T}} ([\text{Cu}(\text{II})]_{\text{T}} = [\text{Cu}(\text{dpba})(\text{H}_2\text{O})^{2+}] + [\text{Cu}(\text{dpba})(\text{OH})^+])$, rate is expressed as follows.

$$\begin{aligned} \text{rate} &= k K K_a [\text{Cu}(\text{II})]_{\text{T}} / (K_a + [\text{H}^+]) \\ &= k_{\text{obs}} [\text{Cu}(\text{II})]_{\text{T}} \\ ([\text{Cu}(\text{II})]_{\text{T}} &= [\text{Cu}(\text{dpba})(\text{H}_2\text{O})^{2+}] + [\text{Cu}(\text{dpba})(\text{OH})^+]) \\ k_{\text{obs}} &= k K_a K / (K_a + [\text{H}^+]) \\ 1/k_{\text{obs}} &= [\text{H}^+ / k K K_a + 1/k K \end{aligned}$$

A plot of $1/k_{\text{obs}}$ vs. $[\text{H}^+]$ was linear and K_a was calculated from a slope and intercept with a value of 5.6 which is smaller than the experimental data, 6.4 but is quite valuable. The results indicate that the reaction mechanism is considerably consistent with the experimental results. The observed rate constants (pseudo-first order) are comparable to other reported results of metalloenzyme models, in which the amide carbonyl is coordinated to the metal. (Collman and Kimura, 1967; Curtis and Sargeson, 1984; Chin et al., 1990) From this result, catalytic ability is not quite different between Lewis acid mechanism or combined one of J. Chin and metal-hydroxide one in this model study. Hydrolysis reaction rate is increased as temperature is raised. From an Arrhenius plots gave ΔH^{\ddagger} , 81 kJ/mol.

When it is compared with nature peptidases, it is helpful to understand the role of metal in enzyme and to verify the mechanism even though the reactivity of our copper complex for the amide hydrolysis is much less than nature enzyme.

4. Conclusion

Amide ligand hydrolysis in the copper complex in which the copper is not coordinated by amide carbonyl has been investigated in acidic condition over the pH range of 5.5 to 6.3. The reaction rate was observed as the first order with copper complex, which means the copper catalyzes the hydrolysis not intermolecularly but intramolecularly. The reaction rate increases as $[H^+]$ is decreased. The copper-hydroxide mechanism is proposed for the amide hydrolysis in the copper complex. The results can be applied to develop new catalysts for peptide bond dissociation in further research.

5. Acknowledgement

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금속착물로 아미드 가수분해 촉매화에 관한연구

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본 연구는 날로 더해가는 오염의 직접간접 원인인 고분자성 제품류의 분해 촉진에 사용될 촉매 개발의 일차적 연구로서, 구리 촉매작용에 의한 아미드 결합의 분해 반응을 수행하였다. 가시광선 스펙트럼의 변화를 측정함으로써 반응을 추적하였다. 아미드 리간드를 포함하는 구리 화합물에서 수용액의 pH의 증가에 따라, 온도의 증가에 따라 아미드의 반응속도가 증가한다. 반응속도는 구리 화합물에 대하여 1차 반응으로 밝혀졌다. 반응의 중간체로 구리-히드록시 화합물이 관여하는 반응 메카니즘을 제시하였다. 분해 반응 메카니즘의 확실한 이해를 통하여 펩티드 결합의 분해 반응에 사용될 좋은 촉매 개발에의 응용이 기대된다.