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Studies on Functional Latices. Catalytic Effects of Histamine-Containing Polymer Latex–Copper(II) Complex on the Oxidation of Ascorbic Acid

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ABSTRACT: The catalytic activity of the histamine-containing polymer latex–Cu(II) complex (abbreviated latex-His–Cu(II)) on the oxidation of ascorbic acid is examined. The latex catalyst shows a so-called Michaelis–Menten type saturation behavior. The Michaelis constants (K_m) and reaction rate constants of the latex catalyst–substrate complex (k_2) are obtained by double reciprocal plots at various temperatures. K_m of the latex-His–Cu(II) system is much larger (about 90 times) than that of the poly(L-histidine)–Cu(II) system because of the electrostatic repulsion between the anionic substrate and the carrier latex. k_2 of the latex system, however, is about 10 times larger than that of poly(L-histidine)–Cu(II), which suggests the cooperative action of the Cu(II)–imidazole complex with neighboring carboxyl groups on the latex surface. Reusability and storage stability of the latex catalyst are also examined.

Most enzymes that act as catalysts in organisms are spherical proteins consisting of repeatedly folded polypeptide chains. In recent years, interest has been shown in studying polymer latex microspheres as enzyme-like hydrolytic catalysts,[1] electrically charged polymer catalysts,[2] and carriers of immobilized enzymes[3,4] and immunoglobulin.[5] It is well-known that cupric ion exists at the active site of some oxidases, such as tyrosinase.[6] To mimic enzymatic oxidation systems, the catalytic behavior of polymer–copper(II) complex in the oxidation reaction has been extensively studied.[7] Pecht et al.[8] found that the oxidation of ascorbic acid was effectively catalyzed by the poly(L-histidine)–Cu(II) complex (PLH–Cu(II)). This complex was homogeneous in water solution and exhibited a Michaelis–Menten type behavior. There is no report on the polymer latex–metal complex as an oxidase-like catalyst. In this paper, we report the enzyme-like catalytic effects of the histamine-containing polymer latex–Cu(II) complex in the oxidation of ascorbic acid.

Experimental Section

Materials. Styrene (St) and acrylic acid (AA) were purified by vacuum distillation. Divinylbenzene (DVB) was washed with 2 N NaOH aqueous solution to remove the inhibitor. Potassium peroxydisulfate (KPS), ascorbic acid, acetic acid, sodium acetate (NaAc), and cupric sulfate were analytical-grade reagents and were used without further purification. Histamine (His) and 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) were obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Doubly distilled water was used in all reactions.

Copolymerization of Latices. Polymer latices were prepared by emulsifier-free copolymerization as follows: 149 mL of water was poured into a three-necked 250-mL round-bottomed flask equipped with a mechanical stirrer, a reflux condenser, and a N_2-inlet tube. The flask was thoroughly filled with pure nitrogen gas kept at 70 °C. A total 28.3 mL of St, 0.62 mL of AA, and 0.25 mL of DVB were poured into the flask, and 105 mg of KPS was finally added as initiator. The mixture was stirred at 240–250 rpm for 5 h while N_2 was continuously passed through the flask. After the copolymerization was complete, the temperature of the solution was raised to 80 °C, and the reflux condenser and N_2-inlet tube were taken away to evaporate unreacted monomers. Two hours later heating and stirring of the solution were stopped. The polymer latex solution was cooled to room temperature, passed through a 120-mesh Nylon filter cloth, and poured into a glass bottle. The latex particles were purified by ion-exchange resin and dialyzed for 1 week against distilled water.

The composition of the polymer latex particles determined from elemental analyses was C, 91.56; H, 7.74; O, 0.70. The amount of carboxyl group in the latex particles was estimated to be 0.22 mmol/g latex. The average diameter of the particle was estimated to be 2500 Å from electron micrographs with a JEM-100CX electron microscope. The dispersion parameter ($\hat{\delta}/\bar{x}$) was calculated to be 0.014 with

$$\hat{\delta} = \left[\sum_{i=1}^{n}(x_i - \bar{x})^2/(n - 1)\right]^{1/2} \qquad (1)$$

where $\hat{\delta}$ is mean square deviation, x_i is the diameter of the particles, and $\bar{x}$ is the average diameter of the particles. Figure 1 clearly shows that the latex particles have excellent monodispersity and a "clean" surface.

Preparation of Latex-Conjugated Ligands and Their Complex with Cu(II). Histamine was coupled to the latex surface by the EDC method[9]: 50 mL of latex suspension (12.2% solids) was mixed with 150 mL of H_2O, and the pH of the mixture was adjusted to 4.2 with 0.1 N HCl aqueous solution. Then 0.79 g of histamine and 1.30 g of EDC were added, and the pH of the solution was kept at 4.2 for 1 h at room temperature. The reaction mixture was stored at 4 °C under continuous stirring. After 56 h, the reaction mixture was centrifuged for 15 min at 15 000 rpm. The latex particles coupled with histamine were then washed with distilled water three times by centrifugation and were finally suspended in 200 mL of H_2O.

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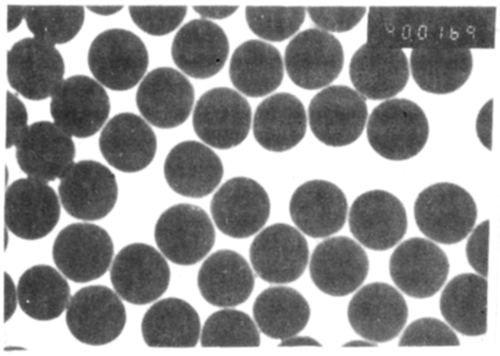

Figure 1. Electron micrograph of polymer latex, ×22 400.

Table I
Kinetic Parameters for the Oxidation of Ascorbic Acid

	15 °C	25 °C	36 °C
$K_m \times 10^3$, M	5.0	3.8	3.2
$V_m \times 10^4$, M·min^{-1}	2.5	3.7	5.4
$k_2 \times 10^{-1}$, min^{-1}	4.3	6.3	9.1

After the coupling reaction, the amount of histamine incorporated into the latex was calculated from elemental analyses to be 0.086 mmol/g latex, and the ratio of the histamine to carboxyl residues was about 1:1.5.

Aqueous $CuSO_4$ solution (0.02 M, 67.9 mL) was mixed with 200 mL of histamine-containing polymer latex suspension at 4 °C for 9 h under stirring. The reaction mixture was then centrifuged at 15 000 rpm for 12 min, and the precipitate was washed with distilled water several times until the supernatant had no further catalytic activity. Finally, the precipitated latex (latex-His–Cu(II) complex) was prepared. The content of Cu(II) on the latex surface was determined by atomic absorption spectrometry with a graphite furnace to be 0.36 mg/g latex.

Kinetic Measurements. The initial oxidation rate of ascorbic acid as substrate was determined by the oxygen-absorption method.[10] Both catalyst suspension and substrate solution were saturated with pure oxygen by bubbling just before the start of the reaction. The initial oxidation rate at various temperatures and different concentrations of substrate were obtained from the change in oxygen absorption volume with time, using a reactor with a constant internal temperature and equipped with an apparatus for determining the volume of the expendable oxygen.

Results and Discussion

Enzyme-Like Behavior of Latex-His–Cu(II). We examined the kinetic behavior of the oxidation of ascorbic acid catalyzed by the latex-His–Cu(II) complex at three temperatures under the conditions of [substrate] ≫ [catalyst]. The relationship between the initial oxidation rate (V_0) and the initial concentrations of substrate ($[S]_0$) is shown in Figure 2a. When the substrate concentration was increased, V_0 first increased and then leveled off. This behavior is characteristic of a Michaelis–Menten reaction pathway (eq 2) involving formation of a catalyst–substrate

$$C + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C{\cdot}S \xrightarrow{k_2} C + P \tag{2}$$

complex. The data were analyzed by the double reciprocal (V_0 vs. $[S]_0^{-1}$) (so-called Lineweaver-Burk plots) with

$$\frac{1}{V_0} = \frac{K_m}{V_{max}}\frac{1}{[S]_0} + \frac{1}{V_{max}} \tag{3}$$

$$V_{max} = k_2[C]_0 \tag{4}$$

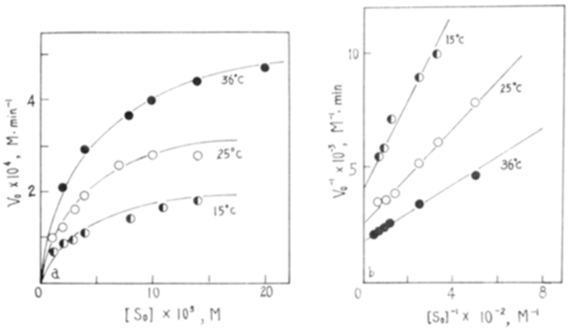

Figure 2. (a) Catalytic effects of latex-His–Cu(II) on the oxidation of ascorbic acid at various temperatures; (b) Lineweaver–Burk plots of (a) in 0.02 M NaAc buffer, pH 4.8, $[C]_0 = 5.88 \times 10^{-6}$ M.

(where $[C]_0$ was the initial concentration of the latex catalyst) and straight lines were obtained (Figure 2b). Kinetic parameters obtained from Figure 2b are summarized in Table I. k_{obsd} for the Cu(II) catalyzed reaction was reported to be 0.16 min^{-1}, and k_2 for the PLH–Cu(II) system was 6.0 min^{-1} at pH 4.3 and 25 °C.[8] Much greater k_2 values observed here show the high catalytic activity of the latex microsphere catalyst, probably the result of the supporting effect of neighboring carboxyl groups on the catalysis by the imidazole ring–Cu(II) complex. The K_m value of the poly(L-histidine)–Cu(II)–ascorbate system was reported to be 4.3×10^{-5} M and is much smaller than the value determined here for the latex-His–Cu(II)–ascorbate system because of the unfavorable electrostatic influence of the surrounding the carboxyl groups in the latter case.

The thermodynamic parameters and reaction rate constant for the oxidation of ascorbic acid catalyzed by latex-His–Cu(II) are calculated by Van't Hoff and Arrhenius equations (5) and (6)[11,12]

$$\Delta G = 2.303RT \log K_m = \Delta H - T\Delta S \tag{5}$$

$$\Delta G^{\ddagger} = 2.303RT \log (KT/hk_2) = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \tag{6}$$

The thermodynamic parameters obtained are summarized in Table II. The negative free energy for the formation of the substrate–catalyst complex is attributed to the increase of entropy. This entropic change may be explained as follows: the complexation of the anionic substrate (pK_1 4.04, pK_2 11.34)[13] with the cationic Cu(II)–imidazole complex induces electroneutralization, and many water molecules around the substrate and catalyst are released resulting an increase in entropy. The higher catalytic activity of latex-His–Cu(II) might be attributed mainly to the decrease in ΔH by the sterically favorable attack of the Cu(II) ion on the fully desolvated substrate which is already bound to the reaction site.

Effects of pH. The effect of pH on the oxidation rate is complicated, since it reflects the sensitivity to pH of both k_2 and K_m. The experimental results are presented in Figure 3. The V_0–pH curve shows a fairly typical enzyme-catalyzed relationship. The initial rate of oxidation changes with increasing pH and reaches a maximum at pH 5.8, which is much lower than the pH optimum for Cu(II) (pH 7.5).[14] The decrease in catalytic activity at high pH can be attributed to the electrostatic repulsion between the anionic carrier latex and the anionic ester. The pH value at which the oxidation rate reaches half its maximum

Table II
Thermodynamic Parameters for the Oxidation of Ascorbic Acid at 25 °C

catalyst	ΔG, kcal·mol^{-1}	ΔH, kcal·mol^{-1}	ΔS, eu	$\Delta G^{\ddagger}$, kcal·mol^{-1}	$\Delta H^{\ddagger}$, kcal·mol^{-1}	$\Delta S^{\ddagger}$, eu
latex-His-Cu(II)	−3.3	3.7	24	17.4	5.2	−40.8

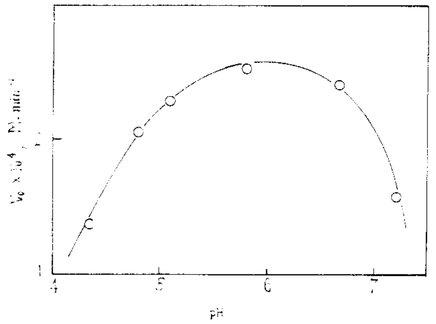

Figure 3. Dependence of oxidation rate on pH at 25 °C, in 0.02 M sodium acetate buffer, $[C]_0 = 5.88 \times 10^{-6}$ M, $[S]_0 = 8 \times 10^{-3}$ M.

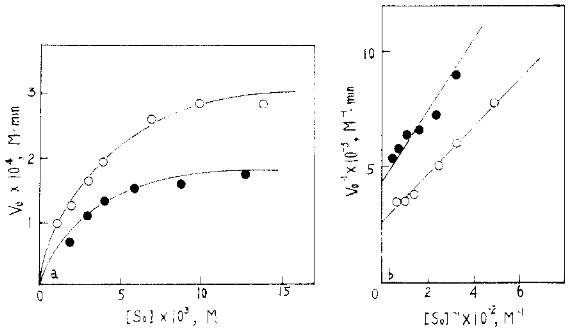

Figure 4. (a) Effects of ionic strength on V_0; (b) Lineweaver–Burk plot of (a) at 25 °C, in 0.02 M NaAc, pH 4.8, $[C]_0 = 5.88 \times 10^{-6}$ M. (○) Latex-His–Cu(II); (●) latex-His–Cu(II) + 0.15 M $NaClO_4$.

Table III
Kinetic Parameters for the Oxidation of Ascorbic Acid at Various Ionic Strengths at 25 °C

	$K_m \times 10^3$, M	$V_m \times 10^4$, M·min^{-1}	$k_2 \times 10^{-1}$, min^{-1}
latex-His–Cu(II)	3.8	3.7	6.3
latex-His–Cu(II) + 0.15 M $NaClO_4$	3.9	2.4	4.0

value (pH 4.6) is shifted to the alkaline region compared with that for PLH–Cu (II).[8] This means that the pH value near Cu(II) is lower than that in the bulk of the solution because of the large amount of residual–COOH on the latex particle surface. A similar tendency was also observed in the hydrolysis of phenyl ester catalyzed by the imidazole-containing polymer latices.[1]

Effect of Ionic Strength. The effect of ionic strength on the initial oxidation rate is shown in Figure 4. Pecht et al.[8] found that the saturation phenomenon disappeared with increasing ionic strength in the PLH–Cu(II) complex catalyzed oxidation of ascorbic acid. They attributed the disappearance of Michaelis–Menten behavior to the decrease of the electrostatic attractive force between the anionic substrate and the cationic catalyst. Michaelis–Menten kinetic behavior, however, can still be observed for latex-His–Cu(II) with increase in ionic strength, except for a slight decrease in the oxidation rate (Table III). This might be attributed to the fact that active groups of the latex catalyst are exposed on the surface of rigid particles, whose conformation cannot be easily changed by a change of environment, which is one of the characteristics of the latex catalyst.

Effects of Temperature. The effects of temperature on the initial rate are shown in Figure 5. When temperature is below 40 °C the initial rate of oxidation increases linearly with the increase in temperature. This is similar to the temperature dependence of the enzymatic reaction. But when the temperature is above 40 °C, the enzymatic reaction rate tends to decrease with increase in temperature because of the denaturation of the enzyme.[15] For the latex-His–Cu(II) system, however, the initial reaction rate continues to increase. This might be another advantageous property of the latex-catalyzed reaction system.

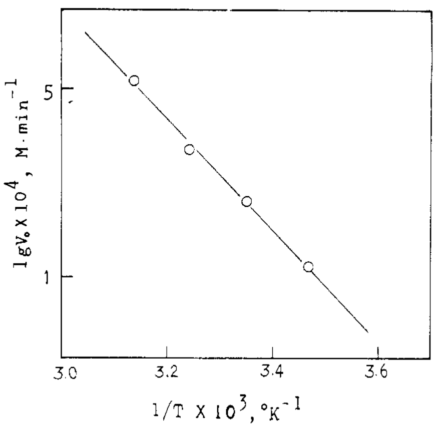

Figure 5. Effects of temperature on V_0 in 0.02 M NaAc, pH 4.8, $[C]_0 = 5.88 \times 10^{-6}$ M, $[S]_0 = 4.0 \times 10^{-3}$ M.

Table IV
Reusability and Storage Stability of Latex-His–Cu(II)[a]

	times of use[b]			storage stability[c]	
	1	2	3	new	110 days
$V_0 \times 10^4$, M·min^{-1}	2.80	2.65	2.73	1.96	1.90

[a] 5.88×10^{-6} M catalyst. [b] 10×10^{-3} M ascorbic acid. [c] 4×10^{-3} M ascorbic acid.

Reusability and Stability of Latex-His–Cu(II). In order to examine the reusability and stability of the latex-His–Cu(II) complex catalyst, we separated the polymer latex catalyst from the reaction mixture by centrifugation, washed it many times, and then suspended it in the same buffer solution. Under the same experimental condition the catalytic activity was examined again. It is observed that the latex-His–Cu(II) catalyst is stable and can be reused many times without losing its activity (Table IV). The dissociation constant of the poly(L-histidine)–Cu(II) complex is also reported to be very small. In the case of homogeneous poly(L-histidine)–Cu(II), however, it is relatively more difficult to recover the catalyst from the reaction solution than from our latex system.

In conclusion, the latex-His–Cu(II) complex shows not only typical Michaelis–Menten catalytic behavior but also high catalytic activity and stability and can be reused many times. Because of the similarity in catalytic behavior of the latex–metal complex to that of a metalloenzyme, further studies on latex–metal complexes will be helpful for the understanding of the catalyst mechanism of metalloenzymes.

Acknowledgment. We are grateful to Professor Norio Ise of Kyoto University for his valuable suggestions and directions.

Registry No. His, 51-45-6; Cu^{II}, 15158-11-9; ascorbic acid, 50-81-7.

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Complex Formation of Poly(ethylenimine) with Sodium Triflate and Conductivity Behavior of the Complexes

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ABSTRACT: Poly(ethylenimine)–$NaCF_3SO_3$ complexes were formed with a polymer repeat unit to salt ratio ranging from 6:1 to 4:1. The complexes change from amorphous to crystalline as the salt concentration is increased. The glass transition temperatures of the amorphous complexes are approximately 15 °C lower than that of the crystalline 4:1 complex. The conductivities of the amorphous complexes are significantly higher than that of the crystalline material. The conductivity plots of all of the complexes follow nearly linear Arrhenius type behavior over the temperature range studied.

Introduction

The potential application of solvent-free polymer–salt complexes as electrolytes in high energy density batteries,[1,2] has stimulated considerable investigation of the fundamental factors governing the behavior of these systems. Most of the focus has been on poly(ethylene oxide) (PEO), which forms complexes with various alkali-metal salts.[2] Vibrational spectroscopy, X-ray diffraction, and EXAFS have provided detailed structural information on PEO–salt complexes.[3,4,5] Free volume and configurational entropy models describe the temperature-dependent and concentration-dependent behavior of the dc conductivity.[2,6] NMR has given insight into the ionic transport mechanism involved in the conductivity behavior of these systems; results indicate that conduction occurs primarily in the amorphous regions of the complexes.[7] The importance of the amorphous phase to ionic transport also is indicated by the enhancement of conductivity seen when a crystalline complex is in a metastable amorphous phase.[8] A set of polymer characteristics that should favor polymer–salt complex formation includes a high concentration of polar groups, polymer flexibility, and low polymer cohesive energy density.[3] Linear poly(ethylenimine) (PEI) ($(CH_2CH_2NH)_n$) has the required closely spaced polar groups and low cohesive energy density. For example, the melting temperature for PEI, 60 °C, compares favorably with that of PEO, 65 °C. In the present research we investigated complex formation between high molecular weight PEI (MW = 100 000) and $NaCF_3SO_3$ (NaTf) and determined the conductivity of these complexes. During the course of our research an independent investigation of low molecular weight PEI (MW ≈ 2000) complexes with NaI was reported.[9]

Experimental Methods

Linear PEI was prepared from poly(ethyloxazoline) (Dow Chemical Co.), average molecular weight 200 000, by acid hydrolysis as described in the literature for a lower molecular weight material.[10] The product was dried at 0.02 torr and 90 °C for 15 h. Ashing of the polymer revealed only 0.02% of inorganic impurities. The product was characterized by a comparison of ^1H NMR spectra,[11] X-ray diffraction patterns,[12] and DSC thermograms[11] with those reported in the literature. Anal. Calcd for $(C_2H_5N)_n$: C, 55.77; H, 11.70; N, 32.50. Found: C, 54.17; H, 11.66; N, 31.19.

Reagent grade methanol (MCB) was dried by reflux over iodine-activated magnesium, followed by distillation under a dry nitrogen atmosphere. Sodium trifluoromethanesulfonate either was commercial reagent grade material or was prepared by neutralizing an aqueous HCF_3SO_3 solution with NaOH to pH 5. All salts were recrystallized from methanol and dried under vacuum at 150 °C. IR spectra of the salt showed no bands due to traces of water at the characteristic frequencies, 3400 and 1610 cm^{-1}.

The polymer–salt complexes were prepared by dissolving stoichiometric quantities of polymer and salt in anhydrous MeOH. After a homogeneous solution was obtained, the methanol was removed under vacuum. The complexes were dried under vacuum at 50–60 °C for 2 days. IR spectra of thin films of the complexes were inspected for bands in the regions around 3400 and 1610 cm^{-1}, arising from trace amounts of water. A broad band at 3300 cm^{-1} due to the N–H stretch of the complexed PEI and smaller bands around 1650 cm^{-1} due to N–H bending partially obscure the water modes and make accurate water detection impossible.

X-ray diffraction measurements were obtained with a diffractometer using Cu Kα radiation. Differential scanning calorimetry (DSC) measurements were performed on a Perkin-Elmer DSC-2 instrument; melting temperatures were obtained at a scan rate of 10 °C/min.

Variable-frequency ac impedance and phase-angle measurements were made over the range 5 Hz to 500 kHz by using a Hewlett-Packard 4800A vector impedance meter. Samples were contained in an airtight cell which was thermostated in an air bath. For these measurements, samples were pressed into the form of disks and sandwiched between Pt electrodes. At temperatures above 60 °C the pressure on the electrodes causes the 5:1 and 6:1 samples to flow, resulting in a short circuit of the cell or changes in the sample thickness. To prevent flow, the samples were pressed onto a Teflon spacer between the electrodes.[13]

Results and Discussion

In a previous study on the acid hydrolysis of poly(*N*-benzoylethylenimine) ($(CH_2CH_2NC(O)C_6H_5)_n$) to give linear PEI, no change was observed in the degree of polymerization from starting polymer to product. On the

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