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Vinyl Polymerization. 377. Polymerization of Methyl Methacrylate by the System of Lysozyme, Copper(II) Ion, and Water

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Vinyl Polymerization. 377.
Polymerization of Methyl Methacrylate by the System of Lysozyme, Copper(II) Ion, and Water

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ABSTRACT

The polymerization of methyl methacrylate (MMA) initiated by the system lysozyme, copper(II) chloride, and water was carried out. The effect of the amount of each component on the conversion of MMA was studied. The polymerization proceeded through a radical mechanism. Urea, known to be a denaturing agent for protein, was able to promote the polymerization. On addition of urea, it was found that the following three reactions took place: (1) promotion of the polymerization of MMA with lysozyme, Cu(II) ion, and water by the unfolding of the third structure of lysozyme, (2) polymerization initiated by urea and Cu(II) ion, (3) polymerization initiated by the S–S bonds of lysozyme and urea.

INTRODUCTION

Previously starch [1], cellulose [2], silk [3, 4], collagen [5], α-amylase [6], ribonucleic acid (RNA) [7, 8], chondroitin sulfate [9], and nylon 3 [10] were found to be able to initiate the radical polymerization of methyl methacrylate (MMA) in aqueous solutions containing trace amounts of metal ion, such as Cu(II) or Fe(III) ion.

The present paper is concerned with the polymerization of MMA by use of hen egg lysozyme, whose exact structure is known. The effect of the denaturalization of lysozyme by urea on the polymerization is dealt with in particular. Besides, new polymerizations of MMA initiated by the S–S bonds of lysozyme and urea and by urea and Cu(II) ion are considered. The initiation mechanisms are discussed in detail.

EXPERIMENTAL

Materials

Lysozyme used in this paper was hen egg lysozyme which was made available by Prof. F. Sakiyama of Institute for Protein Research of Osaka University. It was purified three times by isoelectric precipitation. MMA and other monomers were purified by usual method and redistilled before use. Urea and metal chlorides were of special commercial grade and used without further purification. Water was ion-exchanged and distilled.

Procedures

MMA and other reagents were placed in a tube. The tube was sealed under vacuum after thawing with nitrogen and shaken in a thermostat. After a definite time, the contents of the tube were poured into a large amount of methanol to precipitate the polymer. The polymer collected on the filter was thoroughly washed with methanol. After drying at 80°C under vacuum, the polymer was weighed. Elemental analysis and IR confirmed that the polymer contained no lysozyme and could be regarded as homopoly-MMA. Therefore, no grafted copolymerization of MMA onto lysozyme was observed. Thus, the conversion was calculated by the following equation:

$$\text{Conversion (\%)} = \frac{\text{Wt. of polymer (g)}}{\text{Wt. of MMA added (g)}} \times 100 \qquad (1)$$

The number-average degrees of polymerization ($\overline{P}_{no}$ and $\overline{P}_{nt}$) of poly-MMA obtained in the presence and the absence of lysozyme were calculated by Welch's equation [11] from $[\eta]$ measured at 30°C in benzene. The corrected $\bar{P}_n$ of poly-MMA ($\bar{P}_{nc}$), which was produced by the initiation with the lysozyme system, was calculated according to the previous paper [12].

The surface views of the polymerized system were observed by scanning electron microscopy (Nihon Denshi Co. JSM-35 type). One drop of the reaction system was mounted on a cylindrical brass support with nitrocellulose film, freeze-dried under vacuum, and rendered electrically conductive with a 20-30 nm coating of gold. Microscopy was performed at an accelerating voltage of 15 kV.

RESULTS AND DISCUSSION

Selectivity of Vinyl Monomer

In order to observe the selectivity for vinyl monomer in the polymerization by the system of lysozyme, copper(II) chloride, and water, the polymerizations of four kinds of vinyl monomer were carried out.

As shown in Table 1, the polymerization of MMA was accelerated by lysozyme in the aqueous solution of copper(II) chloride, while polymerizations of styrene (St), acrylonitrile (AN), and methacrylonitrile (MAN) were not.

Proof of Radical Mechanism

A mixture of 3 cm^3 of MMA, 0.1 g of lysozyme, and 10 cm^3 of water containing 0.1 g of copper(II) chloride was shaken at 85°C for

TABLE 1. Vinyl Polymerization with Lysozyme[a]

Lysozyme (g)	Conversion (%)			
	MMA	St	AN	MAN
0	0.36	2.21	0.14	0
0.1	3.84	2.72	0.10	0

[a] Monomer, 3 cm^3; $CuCl_2 \cdot 2H_2O$, 0.1 g; H_2O, 10 cm^3; 85°C; 3 hr.

TABLE 2. Effect of Radical Scavenger on the Polymerization of MMA[a]

Radical scavenger	Conversion (%)
None	3.84
DPPH	0
TPV	0

[a] MMA, 3 cm^3; lysozyme, 0.1 g; H_2O, 10 cm^3; $CuCl_2 \cdot 2H_2O$, 0.1 g; radical scavenger, 0.1 g; 85°C; 3 hr.

3 hr to give the conversion of 3.84%. With addition of 0.1 g of 1,1-diphenyl-2-picrylhydrazyl (DPPH) or 1,3,5-triphenylverdazyl (TPV) as radical scavenger, no formation of poly-MMA was observed (Table 2). Thus, it was concluded that the polymerization proceeded through a radical mechanism.

Alternatively, the radical mechanism was confirmed by the method of copolymerization of MMA with St. The results are shown in Fig. 1.

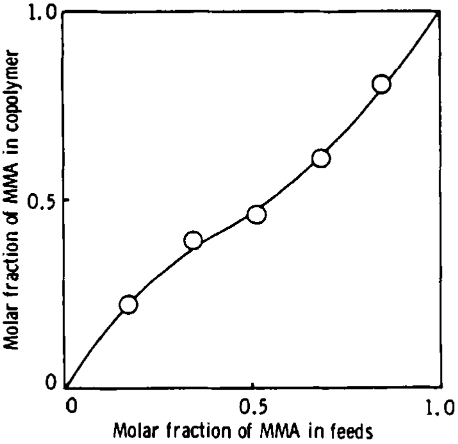


FIG. 1. Composition curve of copolymerization of MMA with St. (MMA + St), 3 cm^3; lysozyme, 0.1 g; $CuCl_2 \cdot 2H_2O$, 0.1 g; H_2O, 10 cm^3; 85°C; 3 hr.

TABLE 3. Activity of Various Metal Chlorides[a]

Lysozyme (g)	Conversion (%) $FeCl_3 \cdot 6H_2O$	$FeCl_2 \cdot (4H_2O)$	$MnCl_2 \cdot 4H_2O$	$ZnCl_2$	$CoCl_2 \cdot 6H_2O$	$CuCl_2 \cdot 2H_2O$
0	1.06	1.20	0.29	0.53	0.40	0.36
0.1	53.36	1.77	0	2.70	0.42	3.84

[a] MMA, 3 cm^3; metal chloride, 5.87×10^{-4} mole; H_2O, 10 cm^3; 85°C; 3 hr.

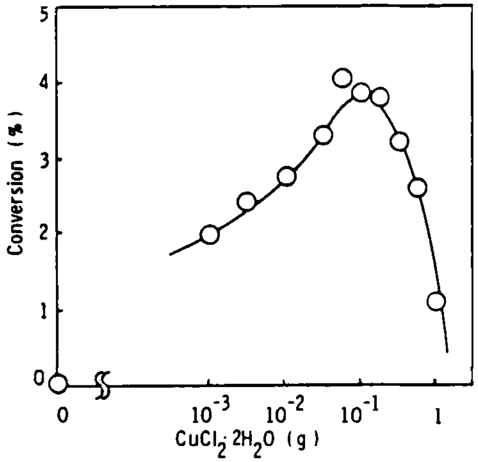


FIG. 2. Conversion of MMA vs. amount of Cu(II) ion. MMA, 3 cm^3; lysozyme, 0.1 g; H_2O, 10 cm^3; 85°C; 3 hr.

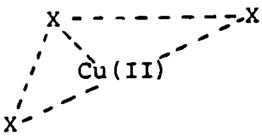
Necessity of Metal Ion for Polymerization

In order to investigate the necessity of metal ion on the polymerization, six kinds of metal chloride were added to the reaction system. From the results, summarized in Table 3, it was concluded that the presence of metal ion was indispensable.

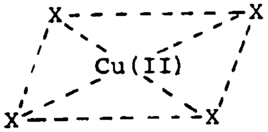
Iron (III), zinc (II) and copper(II) chlorides were active; other kinds of metal chloride were inactive for the polymerization.

Since the activity of Fe(III) ion appeared to be too high to permit study of the kinetics, copper(II) chloride was used in the subsequent polymerizations.

The experimental results on the effect of amount of Cu(II) ion on the conversion of MMA are shown in Fig. 2. At 0.1 g of copper(II) chloride, maximum conversion of MMA was obtained. In comparison with nylon 3, lysozyme required a much larger optimum quantity of Cu(II) ion. In the case of nylon 3, it was only 0.001 g, while in the present case, 0.1 g. The reason may be as follows. Amino or carboxyl groups in the side chains of lysozyme can coordinate with Cu(II) ion more easily than the C=O group of the peptide chain. Therefore, it is difficult to reserve two vacant sites for MMA and water. When three or four coordination sites of Cu(II) ion are filled, as in the structures I and II, Cu(II) ion loses its catalytic activity, as reported previously [10]. Accordingly, in order to hold the activity of lysozyme, a larger quantity of Cu(II) ion should be added.



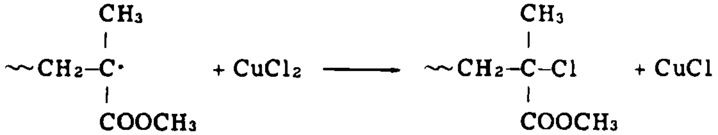
I



II

X: Amino acid residue or –NHCO–

An excess of Cu(II) ion inhibits the polymerization by the reaction:



$$\sim CH_2-\overset{\displaystyle CH_3}{\underset{\displaystyle COOCH_3}{C}}\cdot \quad + CuCl_2 \longrightarrow \sim CH_2-\overset{\displaystyle CH_3}{\underset{\displaystyle COOCH_3}{C}}-Cl \quad + CuCl$$

Necessity of Water for the Polymerization

The relationship between the amount of added water and the conversion of MMA is shown in Fig. 3. It was clear that water was necessary for this polymerization.

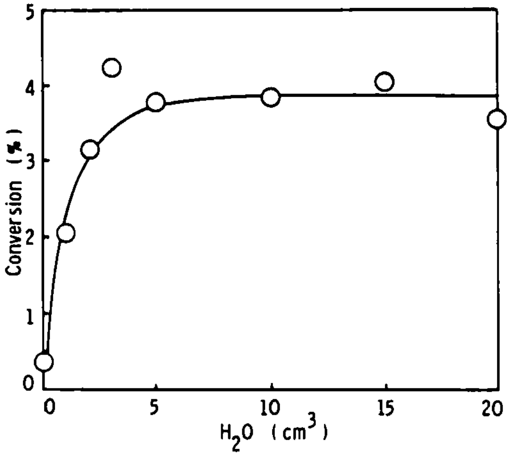


FIG. 3. Effect of the amount of water in the feed on the polymerization of MMA. MMA, 3 cm^3; lysozyme, 0.1 g; $CuCl_2 \cdot 2H_2O$, 0.1 g; 85°C; 3 hr.

Effect of Urea on the Polymerization

Urea is known to be a denaturing agent of protein. The tertiary structure of lysozyme can be unfolded by urea [13, 14]. The unfolding of lysozyme makes easier the formation of hydrophobic areas and the incorporation of MMA, which will be discussed in a later section. Therefore, an increase of the conversion of MMA can be expected on addition of urea. In order to verify this expectation, various amounts of urea were added to the system of 3 cm^3 of MMA and 10 cm^3 of water containing 0.1 g of $CuCl_2 \cdot 2H_2O$ in the presence of 0.1 g of lysozyme. The results obtained are shown in Fig. 4. The conversion of MMA in the presence of lysozyme increased remarkably with the amount of urea in the feed. A similar tendency was also seen in the polymerization of MMA, when α-amylase [6] or nylon 3 [15] was used.

On the other hand, as the lower curve in Fig. 4 shows, the conversion of MMA in the absence of lysozyme increased slightly with the amount of urea; thus urea itself has initiation activity for the polymerization. Accordingly, the increase of the conversion by urea in the system of lysozyme, Cu(II) ion, and water can be explained by unfolding of the tertiary structure of lysozyme and by the polymerization with urea and Cu(II) ion, which is studied precisely in a subsequent section.

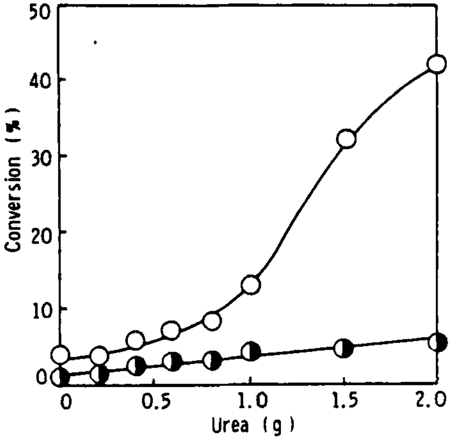


FIG. 4. Conversion of MMA vs. amount of urea: (○) 0.1 g lysozyme; (◑) no lysozyme. $CuCl_2 \cdot 2H_2O$, 0.1 g; H_2O, 10 cm^3; 85°C; 3 hr.

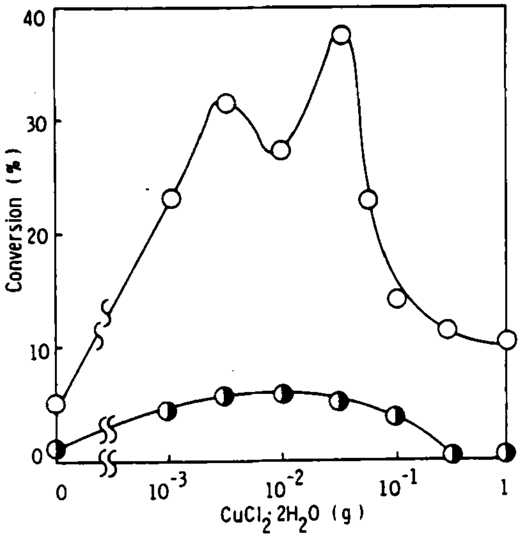


FIG. 5. Influence of lysozyme on the relationship between amount of Cu(II) ion and conversion: (○) 0.1 g lysozyme; (◑) no lysozyme. MMA, 3 cm^3; urea, 1 g; H_2O, 10 cm^3; 85°C; 3 hr.

Relationship between Conversion of MMA and Amount of Cu(II) Ion in the Presence of Urea

In the presence of urea, the effect of the added amount of Cu(II) ion on the polymerization was studied. The results are shown in Fig. 5. In the presence of 1 g of urea, two peaks of conversion were clearly obtained at the amounts of 0.05 g and 0.005 g of $CuCl_2 \cdot 2H_2O$ on addition of 0.1 g of lysozyme, while only one peak of conversion was obtained at 0.01 g of $CuCl_2 \cdot 2H_2O$ without lysozyme. These results suggested that a new type of polymerization took place, in addition to the ordinary uncatalyzed polymerization.

Also in the presence of urea, the overall polymerization proceeded through a radical mechanism. This was confirmed by the copolymerization of MMA with St (Fig. 6).

Polymerization of MMA Initiated with Urea and Cu(II) Ion

As can be seen in Fig. 5, even when lysozyme was absent, some polymerization of MMA took place (up to 5%) in the presence of urea and Cu(II) ion.

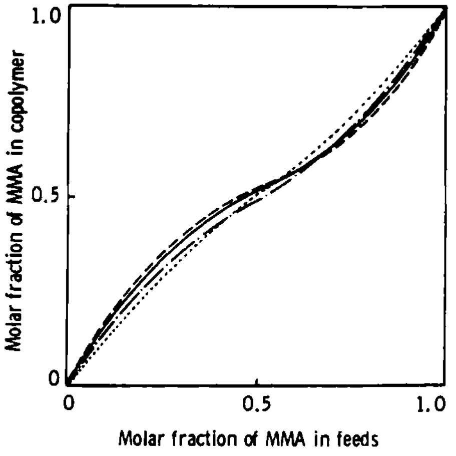


FIG. 6. Copolymerization of MMA with St in the presence of urea: (. . .) 0.1 g $CuCl_2 \cdot 2H_2O$; (——) 0.05 g $CuCl_2 \cdot 2H_2O$; (– · –) 0.005 $CuCl_2 \cdot 2H_2O$; (--) no $CuCl_2 \cdot 2H_2O$. (MMA + St), 3 cm^3; lysozyme, 0.1 g; H_2O, 10 cm^3; urea, 1 g; 85°C; 3 hr.

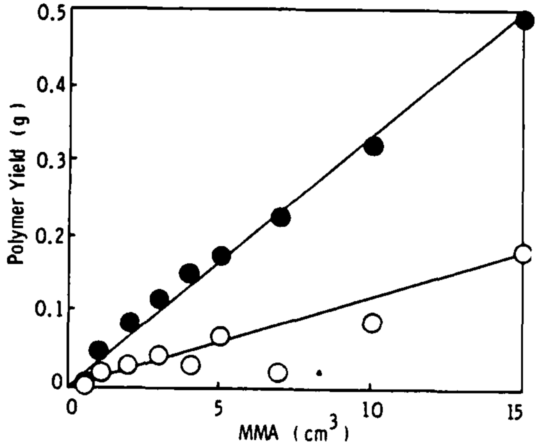


FIG. 7. Effect of amount of MMA on the polymerization initiated with urea and Cu(II) ion: (●) $CuCl_2 \cdot 2H_2O$, 0.001 g; (○) $CuCl_2 \cdot 2H_2O$, 0.1 g. Urea, 1.0 g; H_2O, 10 cm^3; 85°C; 3 hr.

In order to confirm the polymerization, we carried out the reaction of MMA with urea and Cu(II) ion and obtained the results in Fig. 7.

In Fig. 7, the polymer yields in the presence of 0.001 g of $CuCl_2 \cdot 2H_2O$ were larger with 0.1 g. This is due to the inhibiting action of an excess amount of Cu(II) ion, as shown by the lower line in Fig. 5. Therefore, the result shown in Fig. 7 did not indicate the direct effect of Cu(II) ion on the polymer yield.

Granting that the conversion was in a definite relationship with the rate of polymerization R_p, the reaction order with respect to MMA was assumed to be 1.0.

$$R_p = k[\mathrm{MMA}]^{1.0} \qquad (2)$$

In order to estimate the reaction order with respect to urea, the polymerizations shown in Fig. 8 were carried out. From the curve shown in Fig. 8, the reaction order was found to be 0.5.

$$R_p = k'[\mathrm{Urea}]^{0.5} \qquad (3)$$

The reaction order with respect to Cu(II) ion could not be determined, because Cu(II) ion behaved as an initiator and also simultaneously as an inhibitor. The results obtained are shown in Fig. 9.

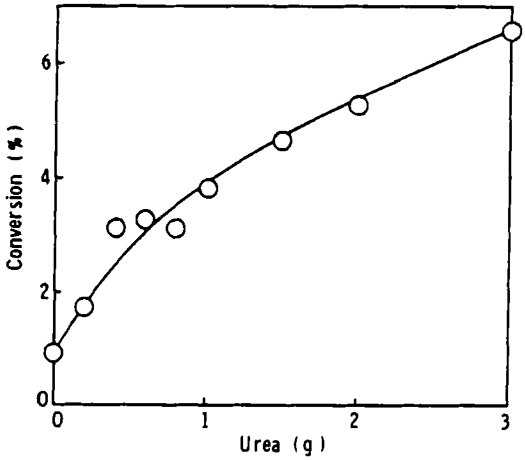


FIG. 8. Conversion of MMA vs. amounts of urea. MMA, 3 cm^3; $CuCl_2 \cdot 2H_2O$, 0.1 g; H_2O, 10 cm^3; 85°C; 3 hr. (Calculated reaction order with respect to urea = 0.49.)

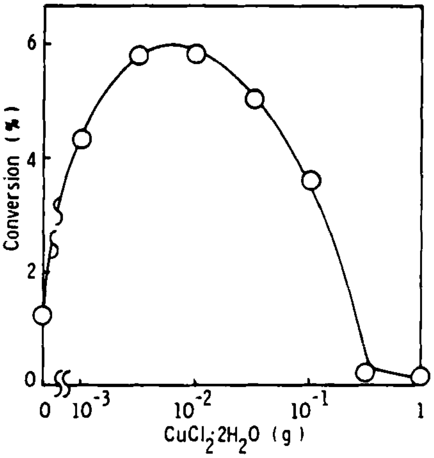


FIG. 9. Conversion of MMA vs. amount of $CuCl_2 \cdot 2H_2O$. MMA, 3 cm^3; urea, 1 g; H_2O, 10 cm^3; 85°C; 3 hr.

Considering that Eqs. (2) and (3) were realized, the polymerization seemed to be an ordinary homogeneous radical polymerization.

Therefore, from the results in Fig. 5, it was suggested that there should be at least four polymerizations I, II, III, and IV by the system of lysozyme, Cu(II) ion, urea and water.

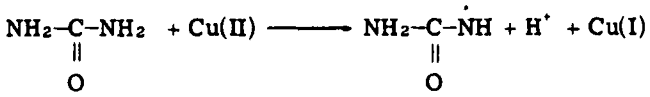
Type I is polymerization of MMA with lysozyme, Cu(II) ion, and water. The polymerization I can be separated into I_f and I_u, where I_f means type I in the absence of urea and I_u is I in the presence of urea. The subscript f indicates a folded structure of lysozyme and subscript u denotes an unfolded conformation.

Type II is polymerization of MMA with urea and Cu(II) ion.

Type III is the "new" type polymerization mentioned above, which is discussed in the later section and is found to be initiated by the reaction of the S–S bond of lysozyme with urea.

Type IV is pure thermal polymerization of MMA. This polymerization may be almost negligible, because the propagating chain, which is initiated in the MMA phase, is immediately scavenged with Cu(II) ion in the water phase by shaking the reaction tube.

The mechanism of the reaction II may be considered as follows:



$$NH_2-\underset{\underset{O}{\|}}{C}-NH_2 + Cu(II) \longrightarrow NH_2-\underset{\underset{O}{\|}}{C}-\dot{N}H + H^+ + Cu(I) \qquad (4)$$

This reaction shall be reported on in detail in the near future.

Interpretation of Figure 5

It is apparent that the upper line in Fig. 5 having the double peak consists of I_u + II + III + IV and the lower curve represents reactions II + IV. Accordingly, on subtracting the conversions of the lower line from those of the upper line, we can obtain the conversions which are the sum of I_u and III. This sum is shown as the solid line in Fig. 10. The two broken lines are the assumed lines for I_u and III, respectively. These dotted lines have been so drawn, that the sum of the two lines agrees with the solid line.

Effects of Amount of Lysozyme on the Conversion of MMA and the Degree of Polymerization of Poly-MMA

In order to observe the effects of the amount of lysozyme on the conversion of MMA and the degree of polymerization of poly-MMA,

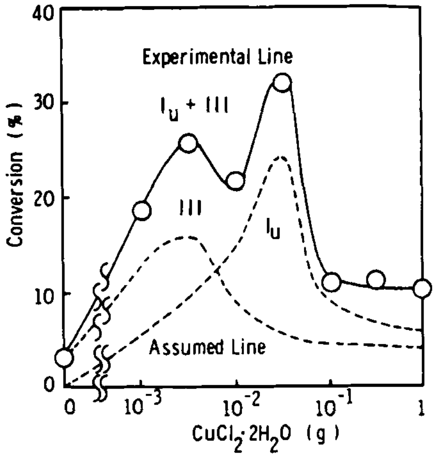


FIG. 10. Synthesis of the experimental conversions (I_u + III) from the assumed conversions of I_u and III.

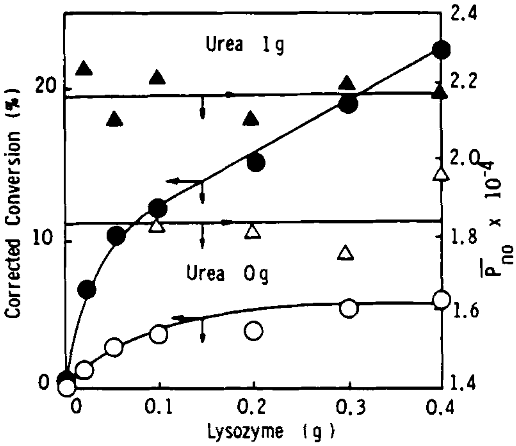


FIG. 11. Effects of the amount of lysozyme on (○, ●) the conversion of MMA and (△, ▲) $\overline{P}_{no}$ of poly-MMA: (● , ▲) urea, 1 g; (○ , △) no urea. MMA, 3 cm^3; $CuCl_2 \cdot 2H_2O$, 0.1 g; H_2O, 10 cm^3; 85°C; 3 hr.

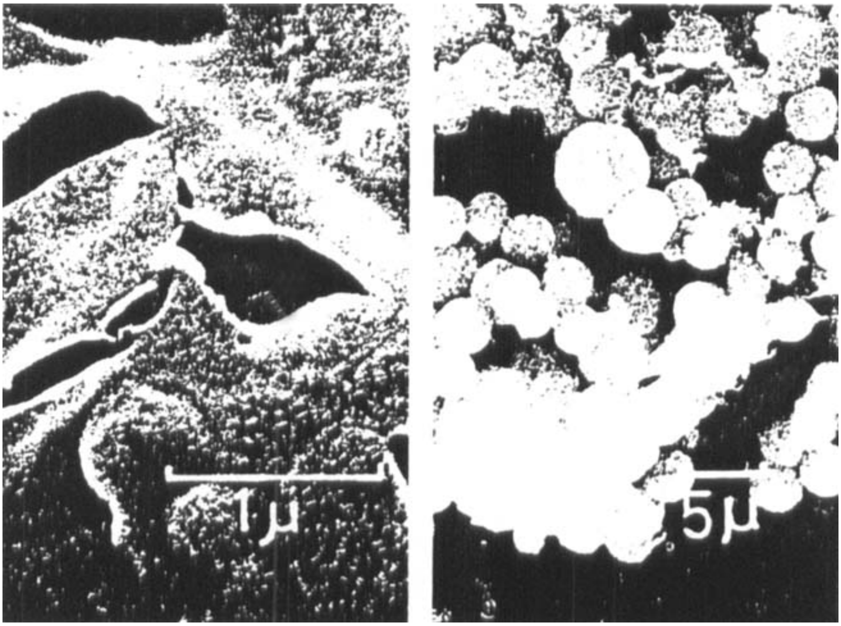


FIG. 12. Surface views of lysozyme: (A) in the absence of urea; (B) in the presence of urea (1 g). (Lysozyme, 0.1 g, was heated in 10 cm^3 of aqueous solution containing 0.1 g of $CuCl_2 \cdot 2H_2O$ at 85°C for 3 hr on standing. The water layer was pipetted out and diluted with water to 100 cm^3.)

the polymerizations of MMA were carried out. The results are shown in Fig. 11. The corrected conversions of the ordinate were calculated by subtracting the conversions in the absence of lysozyme from the overall conversions. As described above, by the addition of urea, the conversion of MMA became larger.

Regardless of the presence or the absence of urea [Eq. (5)], by correlating the logarithms of corrected conversions of MMA with the logarithms of the added amounts of lysozyme was introduced.

$$R_p = k''[\text{Lysozyme}]^{0.5} \qquad (5)$$

On the other hand, $\bar{P}_{no}$ values of poly-MMA in the presence of urea were larger than those in the absence of urea (Fig. 11). $\bar{P}_{no}$ values

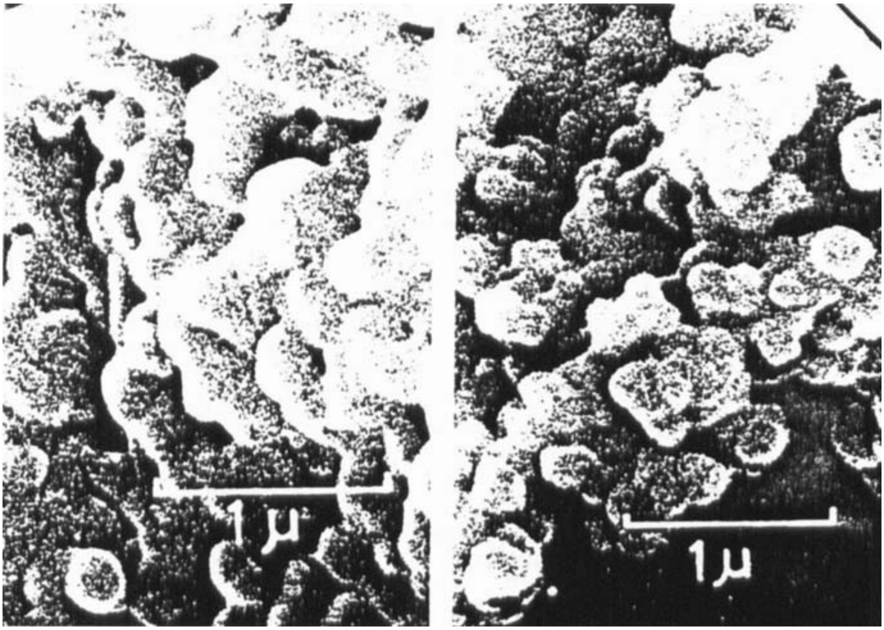


FIG. 13. Surface views of polymerized system: (A) in the absence of urea; (B) in the presence of urea (1 g). (A polymerization of 3 cm^3 of MMA was carried out in 10 cm^3 of aqueous solution containing 0.1 g lysozyme and 0.1 g of $CuCl_2 \cdot 2H_2O$ at 85°C for 3 hr on standing. The water layer was pipetted out and diluted with water to 100 cm^3.)

in both cases were almost independent of the amount of lysozyme in the feed. The reason was considered to be the formation of hydrophobic areas (micelles) by lysozyme. As shall be mentioned just below, the polymerization takes place in these areas.

The formation of the hydrophobic area by lysozyme in the water phase could be observed with aid of electron microscopy. (Figs. 12 and 13). Figures 12A and 12B show the results on the solution of lysozyme and $CuCl_2$ after heating in the absence and the presence of urea, respectively. Figures 13A and 13B show the results after the polymerization of MMA initiated with lysozyme, $CuCl_2$, and water. Hydrophobic areas having distinct shapes, could be formed more easily in the presence of urea than in the absence of urea. It was considered that the more distinct the shape of areas, the more easily MMA could be incorporated and the higher the concentration of MMA in the areas. Therefore, in the presence of urea, the concentration of MMA in the hydrophobic areas became higher. $\bar{P}_{no}$ is correlated with the concentration of MMA in the hydrophobic areas. This is a reason for the higher $\bar{P}_{no}$ in the presence of urea. In the range of 0-0.4 g of lysozyme, the structure of the areas were similar. Hydrophobic areas having similar shapes showed similar $\bar{P}_{no}$ values.

Effect of Amount of MMA on Polymer Yield and Degree of Polymerization of Poly-MMA

Polymerization with 0.1 g of $CuCl_2 \cdot 2H_2O$. Polymerizations were carried out at constant amounts of lysozyme, $CuCl_2 \cdot 2H_2O$ and H_2O in the feed and varying amounts of MMA. The effects of the amount of MMA on the polymer yields in the absence and the presence of urea are shown in Figs. 14 and 15. The curves of overall yield of polymer went upwards. Here, the overall yield in Fig. 14 reflects only the polymerization I_f + IV, while the overall yield in

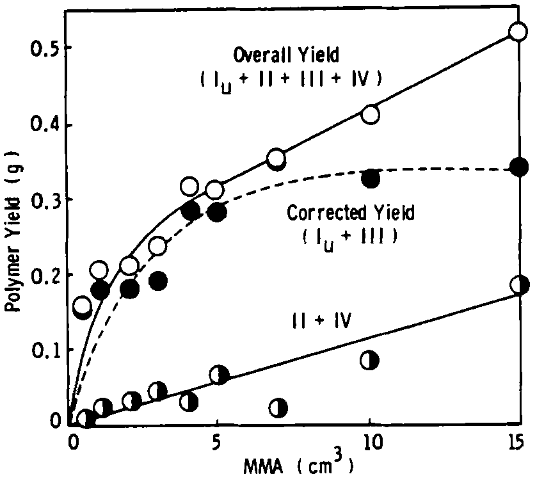


FIG. 14. Effect of the amount of MMA on the polymer yield in the absence of urea: (○ , ●) 0.1 g lysozyme; (◑) no lysozyme. H_2O, 10 cm^3; $CuCl_2 \cdot 2H_2O$, 0.1 g; 85°C; 3 hr.

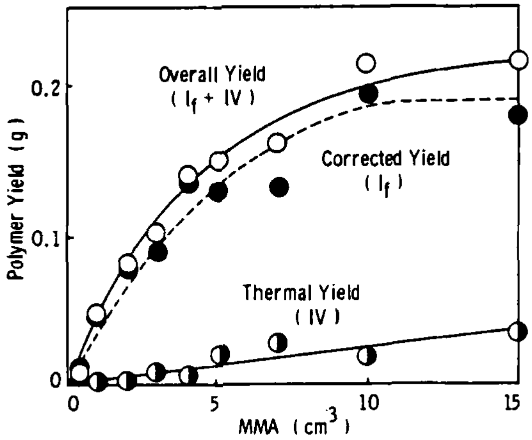


FIG. 15. Effect of the amount of MMA on the polymer yield in the presence of urea: (○ , ●) 0.1 g lysozyme; (◑) no lysozyme. Urea, 1 g; $CuCl_2 \cdot 2H_2O$, 0.1 g; H_2O, 10 cm^3; 85°C; 3 hr.

Fig. 15 represents the sum of the yields by polymerizations I_u, II, III, and IV. In this case, with 0.1 g $CuCl_2 \cdot 2H_2O$, the corrected yield, which was calculated by subtracting the yield by the polymerizations II (Fig. 14) or II + IV (Fig. 15) from the overall yields, increased at first and then became constant, regardless of the presence or absence of urea, as shown by the broken line. The dotted line in Fig. 14 represents the case of I_f, while that in Fig. 15 represents polymerizations I_u + III. In both cases, 0.1 g of $CuCl_2 \cdot 2H_2O$ was used. Accordingly, as assumed in Fig. 10, the polymerization III is considered to be quite a minor reaction.

The corrected yield lines in Figs. 14 and 15 are similar to those which were usually obtained in enzymatic reaction. Accordingly, on regarding MMA as a substrate, the Michaelis-Menten equation, Eq. (6), was applied. As described in the previous paper [12], the rate of reaction in the Michaelis-Menten-Lineweaver-Burk equation [Eq. (7)] is considered to correspond to the initiation rate of the polymerization R_i.

$$\begin{array}{ccccccc} E & + & S & \underset{k_2}{\overset{k_1}{\rightleftharpoons}} & ES & \xrightarrow{k_3} & E + P \\ \vdots & & \vdots & & \vdots & & \vdots \\ \text{Lysozyme} & & \text{MMA} & & \text{Complex} & & \text{MMA}^{\cdot} \\ \text{Cu(II)/H}_2\text{O} & & & & & & \text{(Primary radical)} \end{array} \tag{6}$$

$$\frac{1}{R_i} = \frac{K_m}{k_3[E]}\left(\frac{1}{[S]}\right) + \frac{1}{k_3[E]} \tag{7}$$

$$K_m = (k_2 + k_3)/k_1$$

If we assume that the polymerization is an ordinary radical chain reaction, R_i can be replaced by $R_p/\bar{P}_{nc}$.

$$R_i = R_p/\bar{P}_{nc} \tag{8}$$

$\bar{P}_{no}$ and $\bar{P}_{nt}$ could be measured. From them and the polymer yields, $\bar{P}_{nc}$ was calculated by using the equation reported in the previous paper [12]. The $\bar{P}_{no}$ and $\bar{P}_{nc}$ values obtained in the absence and the presence of urea are shown in Figs. 16 and 17. Thus, R_i^{-1} was coordinated with MMA^{-1}; the results are shown in Fig. 18.

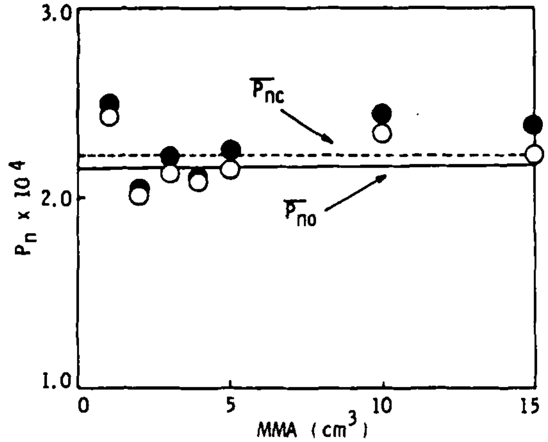


FIG. 16. Effect of the amount of MMA on $\bar{P}_n$ of poly-MMA obtained in the absence of urea. Lysozyme, 0.1 g; $CuCl_2 \cdot 2H_2O$, 0.1 g; H_2O, 10 cm^3; 85°C; 3 hr. Average $\bar{P}_{nt}$ (by polymerization IV): 1.71×10^4.

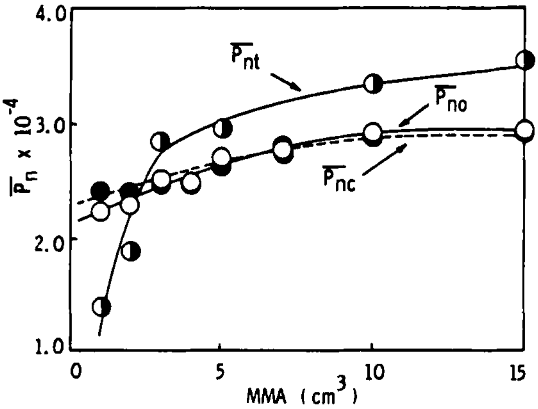


FIG. 17. Effect of the amount of MMA on $\overline{P}_n$ of poly-MMA obtained in the presence of urea. Urea, 1 g; lysozyme, 0.1 g; $CuCl_2 \cdot 2H_2O$, 0.1 g; H_2O, 10 cm^3; 85°C; 3 hr. $\overline{P}_{nt}$ is $\overline{P}_n$ of poly-MMA obtained by polymerizations II and IV.

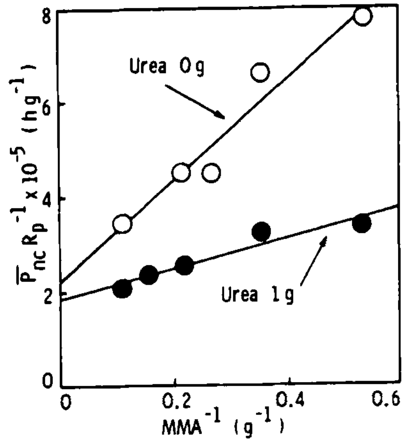


FIG. 18. Application of Michaelis-Menten-Lineweaver-Burk equation to the results shown in Figs. 14-17: (○) no urea; (●) with 1 g urea.

TABLE 4. Parameters of Michaelis-Menten Equation

Type of polymerization	Urea (g)	$k_3[E] \times 10^6$ (g/hr)	K_m (g)
I_u + III	1	5.43	1.71
I_f	0	4.55	4.88

As shown in Fig. 18, the relationship of Eq. (7) was well established. From the intercepts of the ordinate at zero and from the slopes of the straight lines, values of $k_3[E]$ and Michaelis constant, K_m, were estimated (Table 4).

Here, [E] corresponds to the concentration of catalytic sites of lysozyme. Accordingly, [E] in the presence of urea should be much larger than [E] in the absence of urea. Nevertheless, the values of $k_3[E]$ are almost the same, i. e., 5.43×10^{-6} and 4.55×10^{-6} g/hr. No reason for this can be given at the present time.

Polymerization with 0.001 g of $CuCl_2 \cdot 2H_2O$. When 0.001 g of $CuCl_2 \cdot 2H_2O$ was used, the polymerization III is assumed to take place predominantly, according to Fig. 10. Accordingly, in this case the Michaelis-Menten equation cannot be applied. As shown in Fig. 19, the corrected yield was constant, regardless of the amount

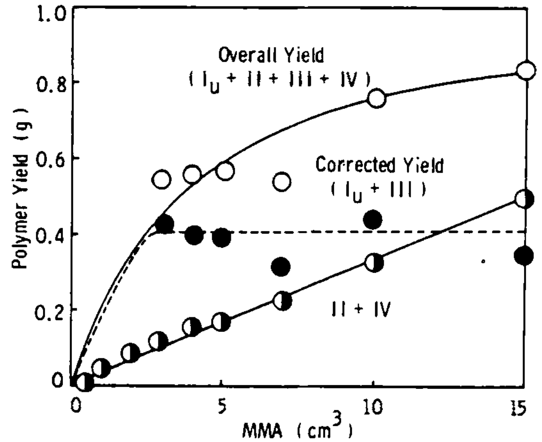


FIG. 19. Effect of the amount of MMA on the polymer yield in the presence of urea. (○, ●) 0.1 g lysozyme; (◑) no lysozyme. Urea, 1 g; $CuCl_2 \cdot 2H_2O$, 0.001 g; H_2O, 10 cm^3; 85°C; 3 hr.

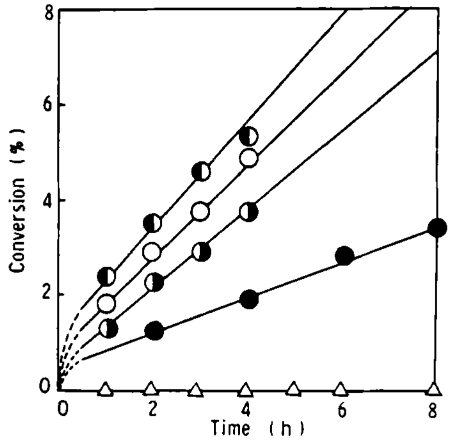


FIG. 20. Time-conversion curves in the absence of urea: (●) 70°C, (◑) 80°C, (○) 85°C, (◐) 90°C; (△) no lysozyme, 70-90°C. MMA, 3 cm^3; lysozyme, 0.1 g unless otherwise noted; $CuCl_2 \cdot 2H_2O$, 0.1 g; H_2O, 10 cm^3.

of MMA. This was due to the saturation of the hydrophobic areas with incorporated MMA. An excess of MMA became useless.

Estimation of Overall Activation Energy

Time-conversion curves were obtained by carrying out the polymerizations at 70-90°C in the absence and the presence of urea (Figs. 20 and 21).

In Fig. 20, the circles indicate the sums of the conversions by the polymerizations I_f + IV, and the triangles the conversions by IV. The conversions by IV were always near to zero. Accordingly, the slopes of the straight lines correspond to R_p of polymerization I_f (in %/hr, which could be changed into g/hr).

In Fig. 21A, the lines consist of the sums of the conversions by I_u + II + III + IV and in Fig. 21B the lines represent conversions by II + IV.

We could calculate the rates of polymerization of MMA which consisting of I_u + III by Eq. (9).

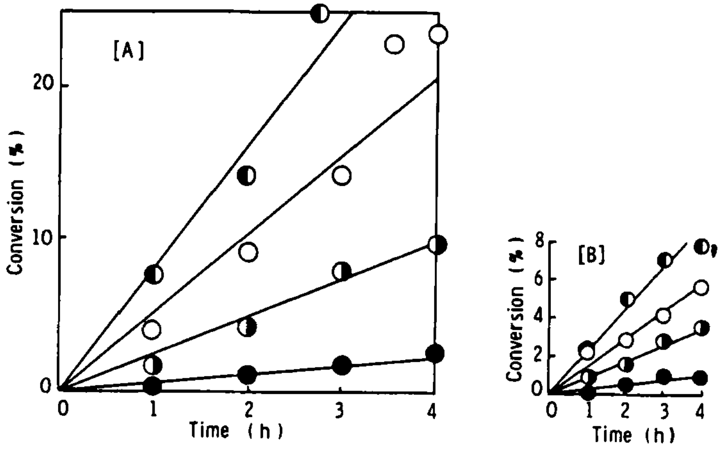


FIG. 21. Time-conversion curves in the presence of urea (A) in the presence of lysozyme (0.1 g) and (B) in the absence of lysozyme. H_2O, 10 cm^3; (•) 70°C; (◑) 80°C, (○) 85°C; (◐) 90°C. Urea, 1 g; MMA, 3 cm^3; $CuCl_2 \cdot 2H_2O$, 0.1 g.

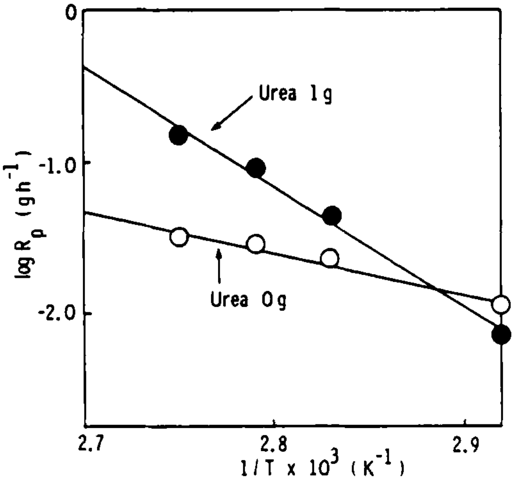


FIG. 22. Arrhenius plots: (•) with urea (1 g); (○) no urea.

TABLE 5. Selectivity of Vinyl Monomer in the Presence of Urea[a]

$CuCl_2 \cdot 2H_2O$ (g)	Lysozyme (g)	Conversion (%)		
		MMA	St	AN
0	0	0.70	2.21	0
0	0.1	4.42	6.27	3.95
5×10^{-3}	0	2.29	2.27	18.80
5×10^{-3}	0.1	31.50	6.25	5.65
5×10^{-2}	0	3.34	1.39	5.20
5×10^{-2}	0.1	37.03	5.73	7.23

[a] Vinyl monomer, 3 cm^3; urea, 1 g; H_2O, 10 cm^3; 85°C; 3 hr.

$$R_p(\text{g/hr}) = \frac{\begin{pmatrix}\text{Overall wt. poly-MMA}\\ \text{obtained in the presence}\\ \text{of lysozyme/g}\end{pmatrix} - \begin{pmatrix}\text{Wt. poly-MMA obtained in}\\ \text{the absence of}\\ \text{lysozyme}\end{pmatrix}}{\text{Polymerization time (hr)}} \tag{9}$$

(As assumed in Fig. 10, the polymerization III is considered to be a much minor one).

By using the values of R_p obtained above, the Arrhenius plots were made (Fig. 22). From the slopes of these straight lines, the apparent overall activation energies in the absence of urea and in the presence of urea were estimated to be 56.1 kJ/mole and 67.0 kJ/mole, respectively. This difference is due to the higher E_a of polymerization III.

Polymerization of MMA by the Polymerization III

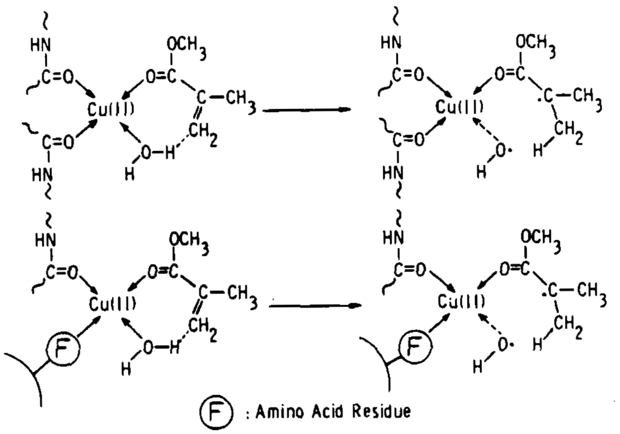
In order to study the new-type polymerization III, MMA, St, and AN were polymerized by the system of lysozyme, Cu(II) ion, water, and urea. The results obtained are shown in Table 5. It was concluded that St and AN could be polymerized. As mentioned before, in the absence of urea, St and AN could not be polymerized. That selectivity for vinyl monomer was not observed suggests the following conclusion: vinyl monomer did not participate in the initiation reaction of lysozyme with urea.

In the cases in which α-amylase and nylon 3 [16] were used, type

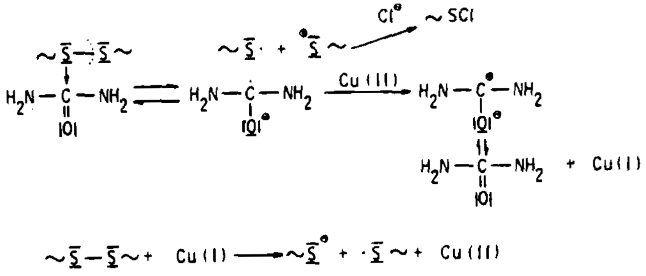
III polymerization was not observed. As is known, a molecule of lysozyme has four S–S bonds, while α-amylase (from Bacillus subtilus) and nylon 3 have no S–S bond. Accordingly, we can suggest that the type III polymerization is based on the reaction of the S–S bond with Cu(II) ion and urea.

Mechanism of Initiation

The polymerization I (the uncatalyzed polymerization) is initiated by the mechanism shown in Eqs. (10), as reported repeatedly [6, 10].



(10)



(11)

The new-type polymerization III was assumed to proceed as shown in Eqs. (11).

As for the initiation with the system of S–S bond, urea, and Cu(II) ion, we have previously reported the vinyl polymerization, using diphenyl disulfide [17].

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