

Vinyl Polymerization. CCCXCVIII. Radical Polymerization of Methyl Methacrylate Initiated with the System of Poly(β -alanine) or α -Amylase and Cu(II) Ion in the Aqueous Solution of Urea*

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ABSTRACT: The polymerization of methyl methacrylate with poly(β -alanine) or α -amylase and Cu(II) ions was carried out at 85°C for 3 h in an aqueous solution of urea. The formation of hydrophobic areas by these macromolecules was observed by scanning electron microscopy. The copolymerization of methyl methacrylate with styrene gave the copolymer with an unusual composition. This fact has been explained on the basis of hard and soft hydrophobic area, and hard and soft vinyl monomer.

KEY WORDS Poly(β -alanine) / α -Amylase / Urea / Hydrophobic Area / Scanning Electron Microscopy /

We found that, urea, which is known to be a denaturing agent toward protein, promotes remarkably the radical polymerization of methyl methacrylate (MMA) initiated with the system of synthesized poly(β -alanine) (nylon-3)¹ or α -amylase² and Cu(II) ions in an aqueous solution.

Following this discovery, we made a detailed study on this promotion in polymerization in which lysozyme³ and glutathione (oxidized type)⁴ were used instead of poly(β -alanine) or α -amylase. It became evident that the following three reactions took place in the presence of urea: (1) promotion by the unfolding of the third structure of lysozyme in the uncatalyzed polymerization of MMA with lysozyme, Cu(II) ion, and water; (2) polymerization initiated by urea and Cu(II) ion; (3) polymerization initiated by the S-S bonds of lysozyme or glutathione and urea.

Poly(β -alanine) and α -amylase having no S-S linkage may be expected to provide a basis for a clearer conclusion regarding the promotion effect of urea on polymerization. The present paper deals with the polymerization of MMA initiated with poly(β -alanine) or α -amylase in the presence of urea.

EXPERIMENTAL

Materials

α -Amylase was a commercial product prepared by Sigma Chemical Company: Bacterial crude type III-A grade (lot. 93C-. 970), 67 units mg⁻¹. (One unit can hydrolyze 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20°C).

Water-soluble (S-type) and water-insoluble (I-type) poly(β -alanine)s were prepared by Breslow's method,⁵ and reprecipitated from the system of methanol and water, and the system of formic acid and water, respectively. The intrinsic viscosities of synthesized poly(β -alanine) measured in 90% formic acid at 30°C are as follows:

Poly(β -alanine) (S), $[\eta]=0.33$

Poly(β -alanine) (I), $[\eta]=0.41$

MMA and other monomers were purified by the usual methods and redistilled before use. Urea and copper (II) chloride 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

* Part CCCXCVII. Refer to ref 4.

tube. The tube was then sealed under vacuum after thawing with nitrogen, and shaken or allowed to stand in a thermostat. After a specified 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. The conversion of MMA was calculated from the weight of the polymerized MMA.

The homopolymer of MMA was isolated by extracting the total polymer with benzene for 50 h. The efficiency of grafting (E.G.) was calculated by

$$\text{E.G.}/\% = \frac{\text{Weight of total poly(MMA)/g} - \text{Weight of homopoly(MMA)/g}}{\text{Weight of total poly(MMA)/g}} \times 100$$

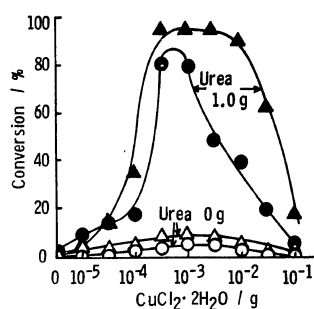


Figure 1. Conversion of MMA vs. amount of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ in the polymerization by poly(β -alanine): MMA, 3 cm^3 ; poly(β -alanine), 0.1 g ; H_2O , 10 cm^3 ; temp, 85°C ; time, 3 h; with shaking; \blacktriangle and \triangle , poly(β -alanine) (I); \bullet and \circ , poly(β -alanine) (S).

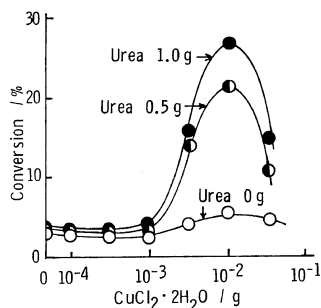


Figure 2. Conversion of MMA vs. amount of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ in the polymerization by α -amylase: MMA, 3 cm^3 ; α -amylase, 0.1 g ; H_2O , 10 cm^3 ; temp, 85°C ; time, 3 h; with shaking; \circ , urea 0 g ; \bullet , urea 0.5 g ; \bullet , urea, 0.1 g .

The number-average degree of polymerization, P_n , was determined from the intrinsic viscosity measured in benzene at 30°C , by using Welch's equation.⁶

RESULTS AND DISCUSSION

Effect of Mass of Cu(II) Ion on the Conversion of MMA

In order to observe the effect of mass of Cu(II) ion on the conversion of MMA in the presence of urea, the polymerizations shown in Figures 1 and 2 were

carried out.

In the polymerization by poly(β -alanine), only one peak of conversion of MMA was obtained, when about $5 \times 10^{-4} \text{ g}$ of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ was used. Furthermore, it was found that the water-insoluble poly(β -alanine) was more effective in bringing about the polymerization than the water-soluble poly(β -alanine), particularly in the presence of urea.

In the polymerization by α -amylase, the maximum conversion was observed at about $1 \times 10^{-2} \text{ g}$ of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, regardless of the presence or absence of urea.

Moreover, because of the urea, an increase in the conversion of MMA was possible. Assuming that poly(β -alanine) and α -amylase contains no S-S bond, such a promotion of the polymerization by urea in the system of poly(β -alanine) or α -amylase, Cu(II) ion, and water may be explained by the unfolding of the tertiary structure of macromolecule and by the polymerization with urea and Cu(II) ion.³

Effect of Amount of Urea on the Conversion of MMA

Figure 3 shows the results obtained in the polymerization by poly(β -alanine) (I). Only 0.3 g of urea was enough to polymerize MMA quantitatively. The E.G. was as high as 55–75% and increased with an increase in the amount of urea. The P_n was constant with or without of urea. This suggests that urea has no influence on the propagating-chain radical of MMA.

The results obtained by α -amylase are shown in Figure 4. The corrected conversion was calculated

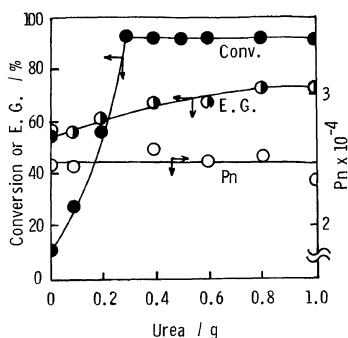


Figure 3. Amount of urea vs. conversion of MMA, E.G., and P_n of homopoly(MMA) in the polymerization by poly(β -alanine) (I): MMA, 3 cm³; poly(β -alanine) (I), 0.1 g; CuCl₂·2H₂O, 1 × 10⁻³ g; H₂O, 10 cm³; time, 3 h; with shaking; ●, overall conversion in the presence of poly(β -alanine) (I); ○, E.G.; ○, P_n of homopoly(MMA).

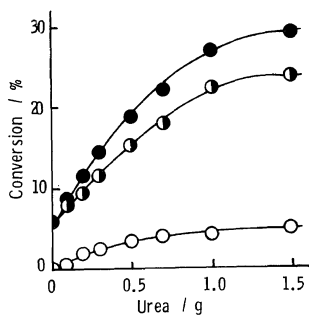


Figure 4. Conversion of MMA vs. amount of urea in the polymerization by α -amylase: MMA, 3 cm³; CuCl₂·2H₂O, 1 × 10⁻² g; H₂O, 10 cm³; temp, 85°C; time, 3 h; with shaking; ○, overall conversion of MMA in the presence of 0.1 g of α -amylase; ●, conversion of MMA in the absence of α -amylase; ●, corrected conversion of MMA.

by subtracting the conversion in the absence of α -amylase from the overall conversion. In comparison with poly(β -alanine) (I), α -amylase required a much greater amount of urea.

Effect of Amount of Macromolecule on the Conversion of MMA

Figure 5 shows the effect of the amount of poly(β -alanine) (I) on the conversion of MMA in the presence of urea. When 0.5 g of urea was used, the conversion of MMA was too high to warrant a discussion of the results. However, when 0.1 g of

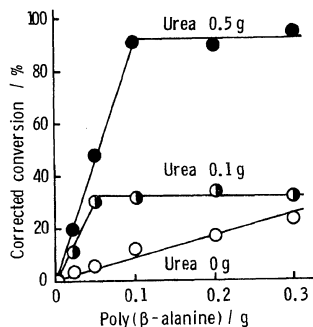


Figure 5. Conversion of MMA vs. amount of poly(β -alanine) (I) in the presence and absence of urea: MMA, 3 cm³; urea, 0–0.5 g; CuCl₂·2H₂O, 1 × 10⁻³ g; H₂O, 10 cm³; temp, 85°C; time, 3 h; with shaking.

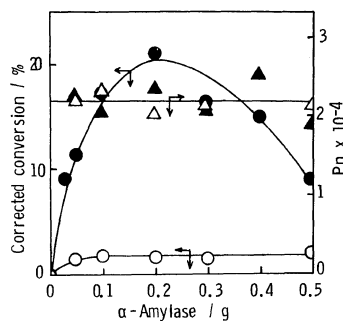


Figure 6. Amount of α -amylase vs. conversion of MMA and P_n of homopoly(MMA), in the presence and absence of urea: MMA, 3 cm³; CuCl₂·2H₂O, 1 × 10⁻² g; H₂O, 10 cm³; temp, 85°C; time 3 h; with shaking; ●, conversion of MMA in the presence of 0.5 g of urea; ○, conversion of MMA in the absence of urea; ▲, P_n of homopoly(MMA) produced in the presence of 0.5 g of urea; △, P_n of homopoly(MMA) produced in the absence of urea.

urea was used, the conversion of MMA was constant at about 32% in the region of 0.05–0.30 g of poly(β -alanine).

The results obtained when α -amylase as a macromolecules was used are shown in Figure 6. When 0.5 g of urea was used, the conversion of MMA increased until 0.2 g of α -amylase was used. However, beyond this, the conversion decreased with an increase in α -amylase. This phenomenon was due to the increase in the molecular entanglement of α -amylase; the formation of the adequate hydrophobic areas (HA) to incorporate MMA

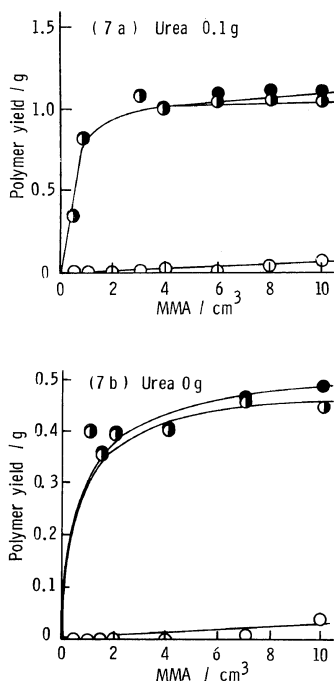


Figure 7. Polymer yield vs. feed amount of MMA in the polymerization by poly(β -alanine) (I): $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 1×10^{-3} g; H_2O , 10 cm^3 ; temp, 85°C ; time, 3 h; with shaking; ●, overall yield in the presence of 0.1 g of poly(β -alanine) (I); ○, yield in the absence of poly(β -alanine) (I); ●, corrected yield.

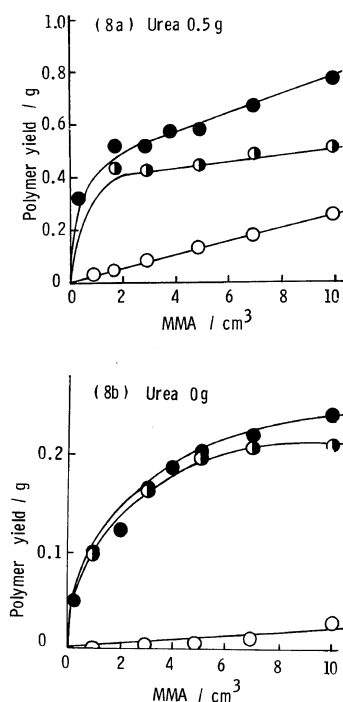


Figure 8. Polymer yield vs. feed amount of MMA in the polymerization by α -amylase: $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 1×10^{-2} g; H_2O , 10 cm^3 ; temp, 85°C ; time, 3 h; with shaking; ●, overall yield in the presence of 0.1 g of α -amylase; ○, yield in the absence of α -amylase; ●, corrected yield.

became difficult as a result of this entanglement.⁷⁻⁹

As described above, the macromolecules of α -amylase or poly(β -alanine) form HA in water.^{3,10} In HA, radical polymerization takes place. HA can be classified as hard or soft HA, according to the degree of hydrophobicity. Similarly, vinyl monomers may be classified as hard or soft monomers, according to their solubility in water. The following new concept is proposed:¹⁰ a vinyl monomer having a certain hardness or softness is most easily incorporated into HA having a corresponding hardness and softness.

The degree of polymerization, P_n , of homopoly(MMA) was constant at about 2.1×10^4 , both in the presence and the absence of urea, and regardless of the amount of α -amylase. These results suggest that α -amylase and urea have no influence on the termination of the propagating-chain radical.

Effect of Amount of MMA on the Yield of

Poly(MMA)

The weight of polymerized MMA was correlated with the feed quantity of MMA both in the absence and the presence of urea. As shown in Figures 7 and 8, in each case the corrected polymer yield became constant, beyond the region in which the yield increased with the quantity of MMA in the feed. This can be explained by the saturation of HA with MMA.³ An excess of MMA was useless. The formation of HA by poly(β -alanine) of α -amylase was confirmed by scanning electron microscopy (Figures 10–13). The lowest line in Figure 7 or 8 shows the polymer yield which is the sum of the yield brought on by the thermal polymerization of MMA and that by the polymerization initiated with urea and Cu(II) ion. The polymerization with urea and Cu(II) ion was studied formerly by us.³ As shown in Figures 7 and 8, when there were no macromolecules, thermal polymerization did not occur. During the polymerization which took place

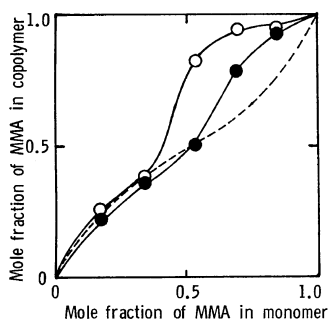


Figure 9. Copolymerization of MMA with St: (MMA + St), 3 cm^3 ; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $1 \times 10^{-3} \text{ g}$; poly(β -alanine) (S), 0.1 g; H_2O , 10 cm^3 ; temp, 85°C ; time, 5 h; on standing; \bullet , urea, 0.5 g; \circ , urea, 0 g. (Dotted line indicates the composition curve which is obtained by the usual radical polymerization.¹¹)

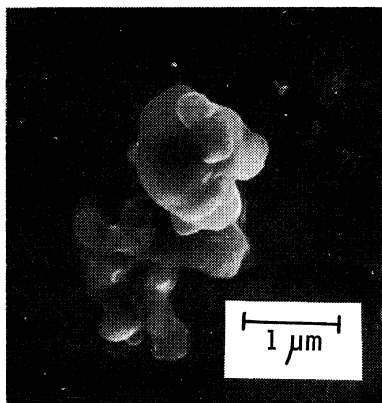


Figure 10. Surface view of poly(β -alanine) (S). A solution of 0.1 g of poly(β -alanine), and $1 \times 10^{-3} \text{ g}$ of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ in 10 cm^3 of H_2O was heated at 85°C for 1 h and diluted to 1 dm^3 .

with shaking, Cu(II) ion easily attacked the propagating-chain radical and inhibited polymerization.

Copolymerization of MMA with Styrene

Copolymerization of MMA with styrene (St) was carried out using poly(β -alanine) (S). The tube containing the reagents was allowed to stand in a thermostat. After polymerization, the upper monomer phase and the lower water phase were pipetted out separately and poured into methanol. The copolymer obtained from the water phase was a product formed by the uncatalyzed polymerization.

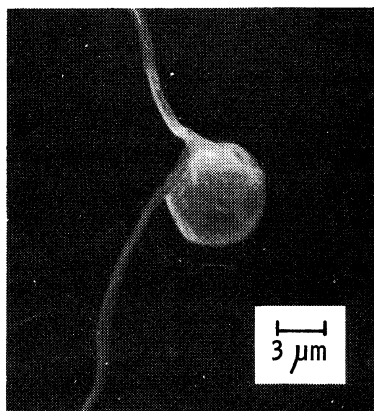


Figure 11. Surface view of the polymerized system by poly(β -alanine) (S). Three cm^3 of MMA and a solution of 0.1 g of poly(β -alanine), $1 \times 10^{-3} \text{ g}$ of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and 0.5 g of urea in 10 cm^3 of H_2O were allowed to stand at 85°C for 20 min. The aqueous phase was pipetted out and diluted to 1 dm^3 .

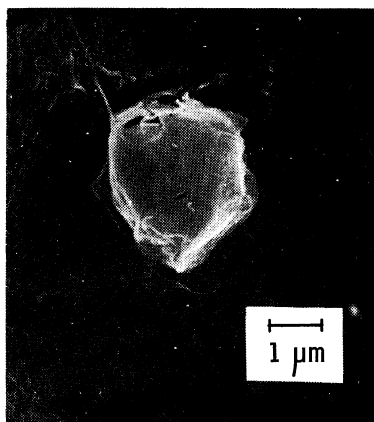


Figure 12. Surface view of α -amylase. A solution of 0.1 g of α -amylase, $1 \times 10^{-2} \text{ g}$ of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and 0.5 g of urea in 10 cm^3 of H_2O was heated at 85°C for 1 h and diluted 1 dm^3 .

As reported previously,³ the uncatalyzed polymerization proceeds mainly in the HA formed by macromolecules. Since the HA formed by poly(β -alanine) is soft, MMA, more easily than St can be incorporated into it. Accordingly, the copolymer produced in the water phase should contain a larger quantity of MMA than the copolymer produced by ordinary radical copolymerization.¹¹

Figure 9 shows the results obtained. The dotted line is the usual radical composition curve. The open and solid circles indicate the compositions of the

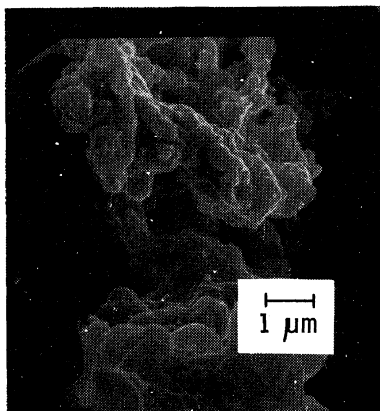


Figure 13. Surface view of the polymerized system by α -amylase. Three cm^3 of MMA and a solution of 0.1 g of α -amylase, 1×10^{-2} g of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and 0.5 g of urea in 10 cm^3 of H_2O were allowed to stand at 85°C for 3 h and diluted to 1 dm^3 .

copolymers obtained from the water phase in the absence and the presence of urea, respectively. As anticipated, the content of MMA in the copolymer is much larger than that in the usual copolymer.

These observations can be explained only by the concept of "hard and soft HA, and hard and soft monomers."¹⁰

Formation of the Hydrophobic Areas

The formation of HA by poly(β -alanine) (S) and

α -amylase was observed using a scanning electron microscope. Figures 10—13 show the results obtained.

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