Grafting of Methyl Methacrylate onto Collagen Initiated by Tributylborane

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Synopsis

The graft copolymerization of methyl methacrylate onto collagen initiated by tributylborane was investigated in aqueous medium. The total conversion, percentage of grafting and efficiency of grafting increased with increasing collagen content. The optimum conditions on the percentage of grafting and efficiency of grafting were determined by varying initiator concentration, monomer concentration, and polymerization temperature. The grafting onto denaturated collagen was also studied. It has been suggested that the grafting onto collagen proceeds by a radical mechanism via a complex of TBB and hydrated collagen.

INTRODUCTION

Chemical modification of naturally occurring polymers by grafting has been attracting attention not only from a practical standpoint, but also from the standpoint of fundamental research. Some aspects of the grafting have been revealed by the studies of several workers.[1–5] The modification of gelatin or collagen by grafting has also been studied, mainly because of its practical importance and interest.[6–10] Potassium persulfate or cerium ion have been used mainly as an initiator for the grafting onto collagen in aqueous media. We have studied the alkylborane-initiated graft copolymerization of methyl methacrylate (MMA) onto cotton,[11] silk,[12] wool,[13] and other proteins.[14] In the present investigation, we studied in detail the grafting of MMA onto collagen at normal temperature using tributylborane (TBB) as an initiator. Electron microscope observation was also carried out on collagen graft polymer. We assumed that the grafting of MMA onto collagen proceeds by radical polymerization initiated by direct interaction between TBB and collagen, most likely via complex formation.

EXPERIMENTAL

Material: Commercial Hide Powder (Wako Pure Chemical, Ltd.) was purified by extraction with acetone in a Soxhlet apparatus for 24 h, followed by washing with methanol and drying under reduced pressure. MMA was purified in the usual manner, bp 44.5°C/95 mm Hg (46°C/100 mm Hg). TBB was used after distillation in a dry box under nitrogen atmosphere, bp 98°C/15 mm Hg (108–110°C/20 mm Hg).[15]

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Grafting: Collagen powder, 0.5 g, was immersed in 10 cm^3 of water at 25°C for 24 h. To this were added 5 cm^3 of MMA and 0.1 cm^3 of TBB. The mixture was shaken in a bath at a constant temperature. The reaction was stopped by pouring the mixture into 200 cm^3 of methanol. The precipitates were filtered, washed with methanol, and dried *in vacuo* to constant weight. The dried precipitates were then extracted with acetone in a Soxhlet apparatus for 72 h. The acetone-soluble portion (homopolymer of MMA) was reprecipitated in methanol. Both the acetone-insoluble portion (graft polymer) and the homopolymer were dried *in vacuo* to constant weight.

Calculation: Total conversion, the percentage of grafting, and the efficiency of grafting were calculated as follows:

$$\text{total conversion (\%)} = \frac{\text{wt poly(MMA) grafted + wt homopolymer}}{\text{wt MMA charged}} \times 100$$

$$\text{percentage of grafting (\%)} = \frac{\text{wt poly(MMA) grafted}}{\text{wt MMA charged}} \times 100$$

$$\text{efficiency of grafting (\%)} = \frac{\text{wt poly(MMA) grafted}}{\text{wt poly(MMA) grafted + wt homopolymer}} \times 100$$

$$\text{total yield (g)} = \text{wt poly(MMA) grafted + wt homopolymer}$$

$$\text{graft yield (g)} = \text{wt poly(MMA) grafted}$$

The Average Molecular Weight of Homopolymer: The molecular weight of homopolymer was determined by viscometric measurement in chloroform at 30°C, based on the relation[16]

$$[\eta] = 4.8 \times 10^{-5} M^{0.8} \ (\text{g·cm}^3)$$

IR Spectra: IR spectra of the backbone and graft polymers were determined from KBr pellets using a Hitachi Model EPI-G2 Spectrophotometer.

RESULTS AND DISCUSSION

Grafting Time

Figure 1 shows the effect of grafting time. Both the total conversion and the percentage of grafting increased with increasing grafting time, and then the efficiency of grafting reached a plateau. These results indicate that for these conditions active sites on collagen powders are not consumed within 3 h. The grafting to collagen powders may be considerably higher than that to collagen film,[6] because the surface area of collagen powder is larger than that of collagen film.

Collagen Content

While the amount of monomer and TBB were kept constant, the amount of collagen was varied in the range of 0.05–0.8 g/20 cm^3. The polymerization of MMA was carried out at 30°C for 2 h. These results are shown in Figure 2. The total conversion, percentage of grafting, and efficiency of grafting increased with increasing collagen content. Homopolymerization conversion, on the other hand,

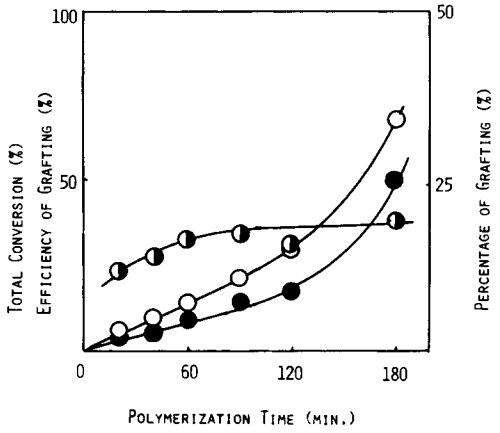


Fig. 1. Effect of polymerization time: collagen, 0.5 g; MMA, 5 cm^3; water, 20 cm^3; TBB, 0.1 cm^3; temp, 37°C. (○) Total conversion (%); (◑) efficiency of grafting (%); (●) percentage of grafting (%).

was not largely dependent on the collagen content except at the high collagen content, in which the polymerization would markedly increase as the solution became viscous. This result could be explained by assuming that the grafting onto collagen is initiated not only by the attack of the primary radicals induced by the reaction of TBB and O_2, but also by the interaction of TBB and collagen.

Initiator Concentration

Figure 3 shows the effects of the initiator concentration. The total conversion and the percentage of grafting have their maxima at 0.1 cm^3/25 cm^3 and 0.07 cm^3/25 cm^3 of TBB concentration, respectively. When the initiator concentration was increased further, both of them increased. It seems that with TBB

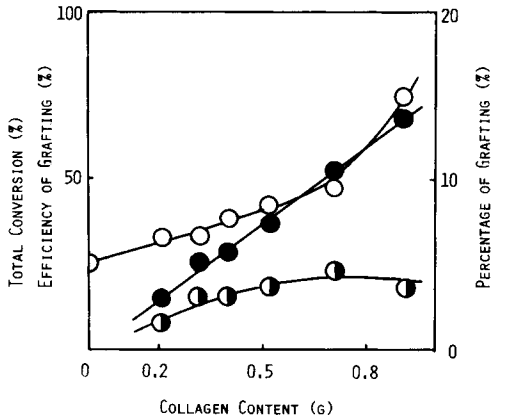


Fig. 2. Effect of collagen content: MMA, 5 cm^3; water, 20 cm^3; TBB, 0.1 cm^3; temp, 37°C; time, 2 h. (○) Total conversion (%); (◑) efficiency of grafting (%); (●) percentage of grafting (%).

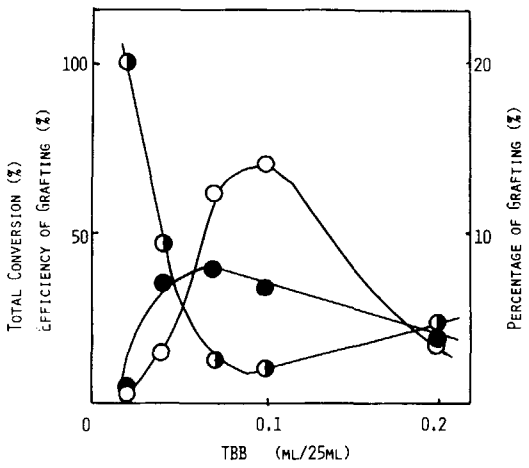


Fig. 3. Effect of TBB concentration: MMA, 5 cm^3; water, 20 cm^3; collagen, 0.5 g; temp, 37°C; time, 2 h. (○) Total conversion (%); (◑) efficiency of grafting (%); (●) percentage of grafting (%).

concentration beyond the maxima both TBB radicals and radicals formed on collagen are wasted by recombination and other termination processes. The efficiency of initiator TBB seems to be considerably lower in this heterogeneous system than in other homogeneous systems.

It has been reported that with concentration of TBB less than 0.02 cm^3/25 cm^3, no polymerization of MMA occurs. Figure 3 shows only graft polymer was obtained with TBB concentration less than 0.02 cm^3/25 cm^3. It is suggested from this result that grafting sites on collagen are introduced by direct interaction of TBB and collagen.

Monomer Concentration

Figure 4 illustrates the dependence of the grafting on the monomer concentration. The graft yield increased with increasing MMA concentration, although total yields decreased at high monomer concentration after having had a maximum at 8 cm^3/25 cm^3. Therefore, the efficiency of grafting increased rapidly with increasing MMA concentration.

Grafting Temperature

As Figure 5 shows, both total conversion and percentage of grafting increase as the polymerization temperature was raised. However, beyond 30°C the efficiency of grafting decreased; beyond 30°C the homopolymerization predominates largely over the graft polymerization.

IR Spectra

The presence of graft polymer on the collagen was confirmed from infrared spectra. The IR spectra of collagen (I), graft polymer (II) (percentage of grafting = 10.6%) and homopolymer (III) are shown in Figure 6. Graft polymer shows

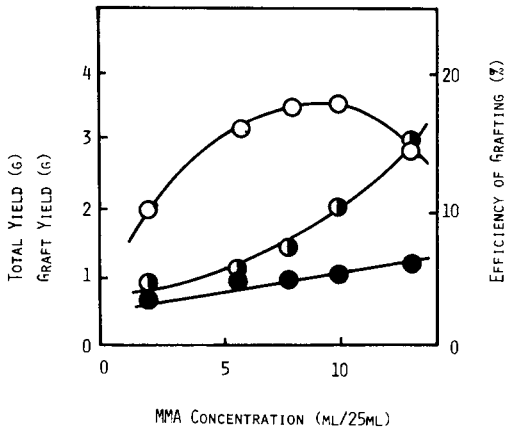


Fig. 4. Effect of monomer concentration: collagen, 0.5 g; TBB, 0.1 cm^3; temp, 37°C; time, 2 h; MMA + water, 25 cm^3. (○) Total yield (g); (●) graft yield (g); (◑) efficiency of grafting (%).

the characteristic absorption band around 1650 cm^{-1} and 1550 cm^{-1} of the amide groups of the collagen. Moreover, graft polymer has the additional absorption bands as compared to the original collagen, at 1750 cm^{-1} (C=O stretching), at 1240 cm^{-1} and 1150 cm^{-1} (C—O stretching), and at 1040, 840, 750, and 700 cm^{-1} (characteristic of methacrylates).

Denaturation of Collagen

It is well known that collagen has a structure which is twisted with three polypeptide chains through hydrogen bonds and can be denatured to random-coiled gelatin by heating, due to breakage of these hydrogen bonds.

Collagen powders were heated at 40°C, 60°C, and 100°C in water for 10 h. Onto this denatured collagen, the grafting of MMA was carried out. No change was observed in the graft polymer on collagen heated both at 40°C and at 60°C.

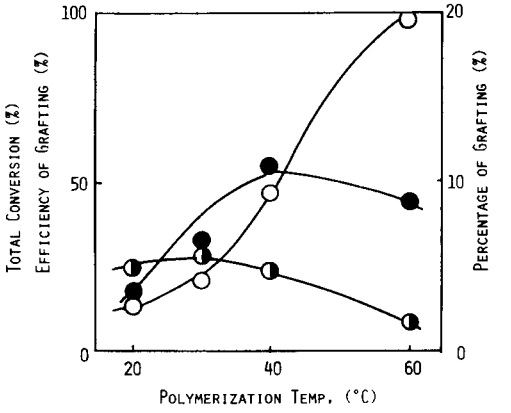


Fig. 5. Effect of polymerization temperature; collagen, 0.5 g; MMA, 5 cm^3; water, 20 cm^3; TBB, 0.1 cm^3; time, 2 h. (○) Total conversion (%); (◑) efficiency of grafting (%); (●) percentage of grafting (%).

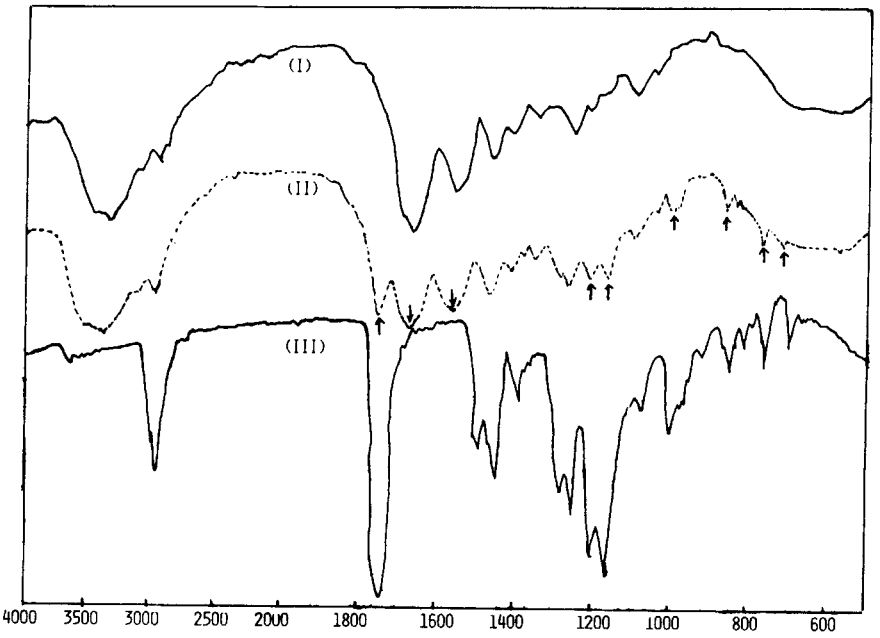


Fig. 6. Infrared spectra: collagen (I), collagen graft polymer (II) (percentage of grafting = 10.6%), and PMMA (III).

The results obtained in the graft polymer heated at 100°C are shown in Figure 7. As the figure shows, the percentage of grafting decreases with increasing thermal treatment time.

Electron Microscope Scanning

The electron microscope scan of grafted collagen powder is shown in Figure 8. When viewed in the electron microscope scan, PMMA appears chemically bonded to the surface of collagen powder.

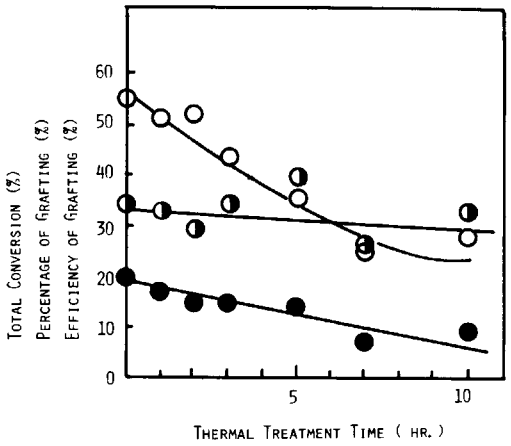
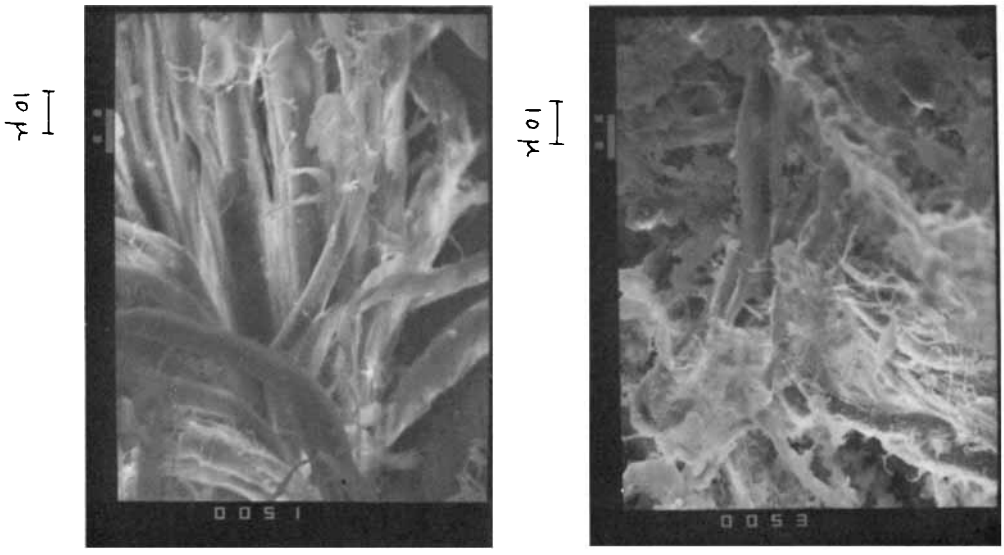


Fig. 7. Effect of thermal treatment (100°C); polymerization conditions: collagen, 0.5 g; MMA, 5 cm^3; water, 20 cm^3; TBB, 0.1 cm^3; temp, 37°C: time, 90 min. (○) Total conversion (%); (◑) efficiency of grafting (%); (●) percentage of grafting (%).



[A] [B]

Fig. 8. The electron microscope scan (× 1000): collagen [A] and collagen graft polymer [B].

Grafting Mechanism

It is possible that grafting sites on collagen are formed by radical chain transfer. If so, it is expected that the average molecular weight of homopolymers initiated by this system decreases with increasing collagen content. Figure 9 shows the relationship between the collagen content and the average molecular weight of homopolymers. As the collagen content increases, the molecular weight in-

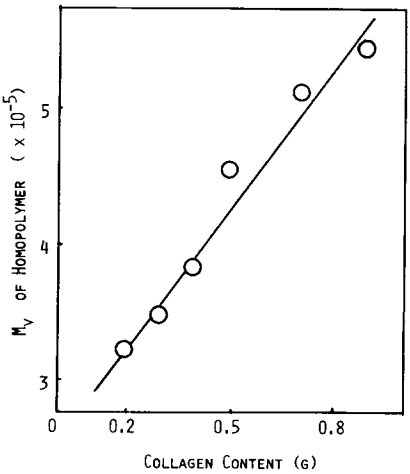
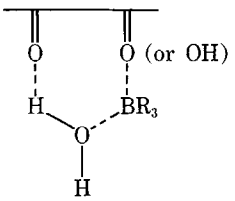


Fig. 9. Relationship between average molecular weight of homopolymer and collagen content.

creases. This result indicates that the formation of grafting sites on the collagen is independent of the chain transfer by macroradicals.

Previously,[17] we showed that the polymerization initiated by TBB was accelerated by the presence of glycine, alanine, glutamic acid, and aspartic acid and that some interaction, to promote the radical-forming decomposition of TBB, must exist between TBB and amino acids. The presence of water is essential to the grafting onto collagen as onto other proteinous materials. No grafting occurred in the usual organic solvents, although TBB can initiate the polymerization of MMA in these organic solvents. As mentioned above, it seems that initially the water adsorbs on the surface of collagen, and then grafting sites are formed by direct interaction between TBB and hydrated collagen, most likely via the complex



The high-dimensional structure of collagen seems also important, as the grafting onto denatured collagen was less efficient and no grafting occurred onto structurally flexible polypeptides such as poly(glutamic acid).[18] These results may be explained on the basis of change in the conformation of collagen to gelatin; the surface areas of denatured collagen, which are exposed to TBB and water, become smaller by extreme folding of a chain rather than in the case of high-dimensional structure.

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