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The Trialkylborane-initiated Graft Copolymerization of Methyl Methacrylate onto Hemoglobin[1]

Koichi KOJIMA, Susumu IWABUCHI, and Kuniharu KOJIMA*,
Department of Applied Chemistry, Faculty of Engineering, Chiba University, Yayoi-cho, Chiba
and Niro TARUMI
Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, Kanda-Surugadai, Chiyoda-ku, Tokyo

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The graft copolymerization of methyl methacrylate by trialkylborane onto hemoglobin has been studied at 37°C. In aqueous media, graft copolymers were obtained in the form of a light brown powder or granules, while no grafting occurred in organic solvents, such as cyclohexanone, *n*-hexane, tetrahydrofuran, and toluene. The presence of water seems to be essential to the grafting. The hydrogen peroxide-decomposing property of hemoglobin was well preserved in the graft copolymers so obtained. The mechanism of the initiation is discussed.

Since the pioneering works by Furukawa *et al.*[2] and by Kolesnikov and Klimentova,[3] it has been shown that trialkylboranes can initiate the polymerization of various vinyl monomers in the presence of oxygen or oxygen-containing compounds.[4-9] On the other hand, some reports on the preservation of blood by chemical treatment have appeared. Suzuki and Hachimori[10] tried to protect blood by the reaction of aldehyde with hemoglobin in vain. Kondo[11] disclosed that blood can be stabilized by enclosing or "wrapping" it with colloidal gelatin.

We have previously described the cocatalytic effects of pyridine and its derivatives on the polymerization of methyl methacrylate (MMA) by tri-*n*-butylborane (Bu_3B) in organic solvents.[12] We have also studied the graft copolymerization of vinyl monomers

* To whom inquiries should be addressed.

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by trialkylborane initiators onto collagen,[13] proteins, and fibers.[14] In another previous paper,[15] we reported the graft copolymerization of MMA by Bu_3B in blood; MMA was found to be grafted onto blood components in the following order:

blood cells > hemoglobin > blood plasma.

In order to elucidate the MMA-grafting in blood, we tried to graft MMA by Bu_3B directly onto hemoglobin. This paper will report some interesting results on the subject.

Experimental

Materials. *Methyl Methacrylate*: One liter of commercial MMA was washed with three 100-m*l* portions of a saturated sodium hydrosulfite solution in a separatory funnel, and then with three 100-m*l* portions of a 20% sodium chloride solution. The MMA so washed was allowed to stand over silica gel overnight and then filtered and distilled in a nitrogen atmosphere under reduced pressure; bp 46°C/100 mmHg.[16]

Tri-n-butylborane (Bu_3B): Commercial Bu_3B (Callery Chemical Co., USA) was distilled under nitrogen just before use; bp 108—110°C/20 mmHg.[17]

Commercial *hemoglobin* and an isotonic sodium chloride solution were used without further treatment. All the *other materials used* were purified in the usual manner and were distilled just before use.

Graft Copolymerization. *Typical Procedure*: A mixture of 0.5 g of hemoglobin and 10 m*l* of an isotonic sodium chloride solution was placed in a stoppered glass tube (inner volume: *ca.* 60 m*l*). In another, smaller glass tube we added 0.10 m*l* of Bu_3B to 5.0 m*l* of MMA. This mixture was immediately poured into the first glass tube. Then, the glass tube was shaken in a thermostatted shaking apparatus at 37°C. The reaction was stopped by pouring the mixture into 200 m*l* of methanol. The precipitate was filtered, washed with methanol, and dried *in vacuo* to a constant weight. The dry precipitate was extracted with acetone in a Soxhlet extractor for 50—80 hr. The acetone-soluble extracts were reprecipitated with methanol to yield a homopolymer. Both the acetone-insoluble residue (graft copolymer) and the homopolymer were dried *in vacuo* to constant weights.

The infrared speatra were obtained with a Hitachi Model EPI-3T spectrophotometer.

Calculation

The total conversion, the percentage of grafting, and the efficiency of grafting were calculated as follows:

$$\text{total conversion} = \frac{\text{weights of poly (MMA) grafted and homopolymer}}{\text{weight of MMA charged}} = \frac{II+III}{I}$$

$$\text{percentage of grafting} = \frac{\text{weight of poly (MMA) grafted}}{\text{weight of MMA charged}} = \frac{II}{I}$$

$$\text{efficiency of grafting} = \frac{\text{weight of poly (MMA) grafted}}{\text{weights of poly (MMA) grafted and homopolymer}} = \frac{II}{II+III}$$

where

I: weight of MMA charged

II: (weight of the acetone-insoluble component) minus (weight of the backbone polymer)

III: weight of the acetone-soluble component (homopolymer)

The hydrogen Peroxide-decomposing Properties of hemoglobin and of the graft copolymer were determined as follows: To 50 m*l* of a 1% hydrogen peroxide solution were added 2 g of hemoglobin or the graft copolymer obtained from 2 g of hemoglobin. The mixture was well stirred. After 1/2, 1, 2, 3, and 5 hr, 1.0 m*l* portions of the hydrogen peroxide solution were taken up by means of a syringe and titrated with a 0.1 N potassium permanganate solution.

Results and Discussion

Effects of Solvents. The graft copolymerization of MMA by Bu_3B onto hemoglobin was carried out at 37°C in various solvents, both in aqueous and organic solvents. The results are listed in Table 1. The percentage of grafting was 7.8 in the isotonic sodium chloride solution and 12.1 in water, while no weight increase of the final product was observed in cyclohexa-

Table 1. Grafting of methyl methacrylate onto hemoglobin

Run No.	Hemoglobin (g)	Solvent	Bu_3B (m*l*)	Total yield (g)	Total conversion (%)	Weight of homopolymer (g)	Weight of graft copolymer (g)	Weight increase of hemoglobin (%)	Percentage of grafting (%)	Efficiency of grafting (%)
001	0.48	W	0.10	4.77	91.0	2.98	1.05	117	12.1	13.3
101	0.53	ISC	0.10	4.92	93.2	3.08	0.90	69	7.8	8.4
102	0.53	ISC	—	0.45	—	—	0.43	—	—	—
601	0.50	HX	0.10	2.90	51.0	2.41	0.47	—	—	—
602	0.50	CHN	0.10	1.51	21.5	1.03	0.47	—	—	—
603	0.50	THF	0.10	0.53	0.6	0.04	0.47	—	—	—
604	0.50	TOL	0.10	1.58	23.0	1.15	0.43	—	—	—

Grafting conditions: Methyl methacrylate: 5.0 m*l*; Solvent: 10 m*l*; 37°C; 2 hr W: water; ISC: isotonic sodium chloride solution; HX: *n*-hexane; CHN: cyclohexanone; THF: tetrahydrofuran; TOL: toluene.

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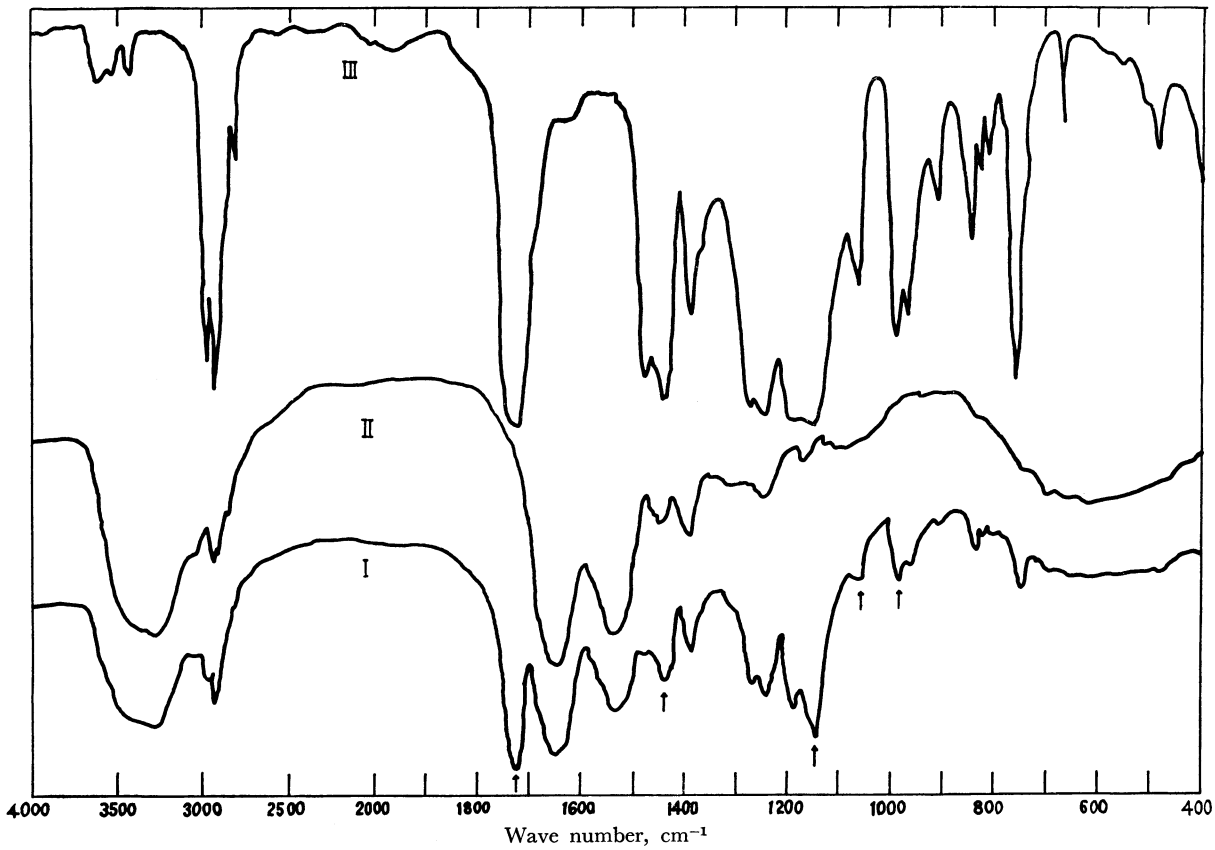

Fig. 1. Infrared spectra of graft copolymer, hemoglobin, and poly (MMA).
I: graft copolymer; II: hemoglobin; III: poly (MMA).

none, *n*-hexane, tetrahydrofuran (THF), and toluene. This means that, in the presence of water only, MMA was grafted by Bu_3B onto hemoglobin. Similar results were also obtained in the graft copolymerization of MMA by Bu_3B onto proteins, such as albumin and casein.[14]

When the graft copolymerization of MMA by Bu_3B onto hemoglobin was carried out in aqueous media, the acetone-soluble parts of the products were obtained in the form of a light brown powder or granules. The infrared spectra are given in Figure 1. The spectrum of the acetone-insoluble part (I in Fig. 1) showed characteristic absorption bands at 1730, 1450, 1150, and 990 cm^{-1}. Since none of these bands were seen in the spectrum of hemoglobin (II), while all of them were found in the spectrum of poly (MMA) (III), the bands may be assigned to the MMA-grafted hemoglobin. The acetone-insoluble parts may, therefore, be believed to be graft copolymers.

Figure 2 shows an electron micrograph of the acetone-insoluble part (graft copolymer).

For purposes of comparison, the same grafting procedure was done in the absence of Bu_3B. The spectra of the acetone-insoluble parts entirely agreed with that of hemoglobin (II), which means that no grafting occurred in the absence of Bu_3B.

Both di-*n*-butylzinc and the well-known benzoyl peroxide/dimethyl-*p*-toluidine system were incapable of initiating the graft copolymeriaztion of MMA onto hemoglobin under the same conditions. This would suggest that the graft copolymerization of MMA onto hemoglobin is specific for tri-*n*-butylborane under these conditions.

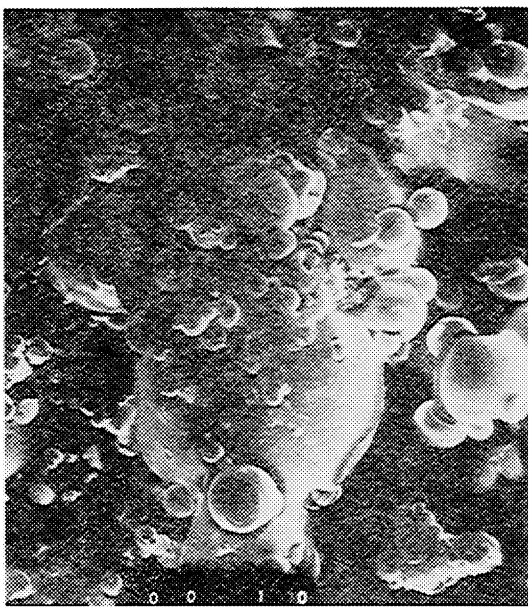

Fig. 2. An electron micrograph of the acetone-insoluble part (graft copolymer), ×500, (viewed in a JELCO JSM-U3 electron microscope).

Reaction Time. The effects of the reaction time on the grafting were also studied. Figure 3 indicates that the grafting proceeded very rapidly in the initial

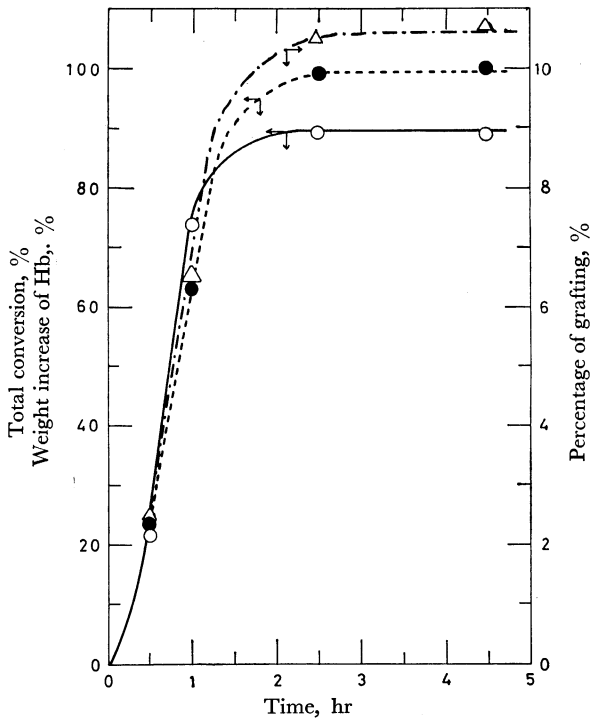

Fig. 3. Effects of reaction time on grafting.
(○): total conversion; (●): weight increase of hemoglobin;
(△): percentage of grafting;
Hemoglobin: 0.5 g; MMA: 5.0 m*l*; Bu_3B: 0.10 m*l*;
Solvent: 10 m*l*; 37°C.

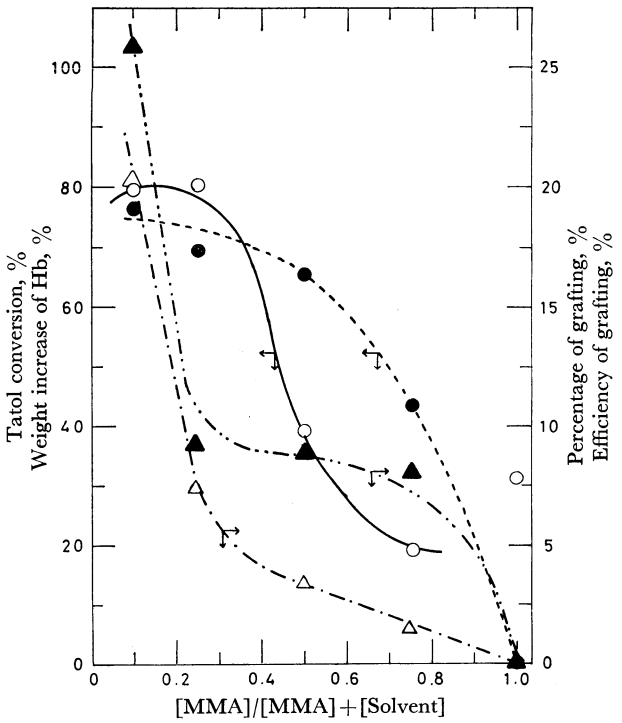

Fig. 4. Effects of monomer concentration.
(○): total conversion; (●): weight increase of hemoglobin;
(△): percentage of grafting; (▲): efficiency of grafting.
Hemoglobin: 0.5 g; (MMA + solvent): 20 m*l*; Bu_3B:
0.10 m*l*; 37°C; 2 hr.

stage and then reached the saturation point in about an hour at 37°C.

Concentration of MMA. The concentration of MMA influenced the grafting (Figure 4). The total conversion was minimal when the concentration of MMA was 70—80%. The weight increase, the percentage of grafting, and the efficiency of grafting decreased with the increase in the concentration of MMA, so that at last no grafting was observed when the concentration of MMA was 100%, that is, in MMA itself and in the absence of water. This also shows that water is essential to the grafting.

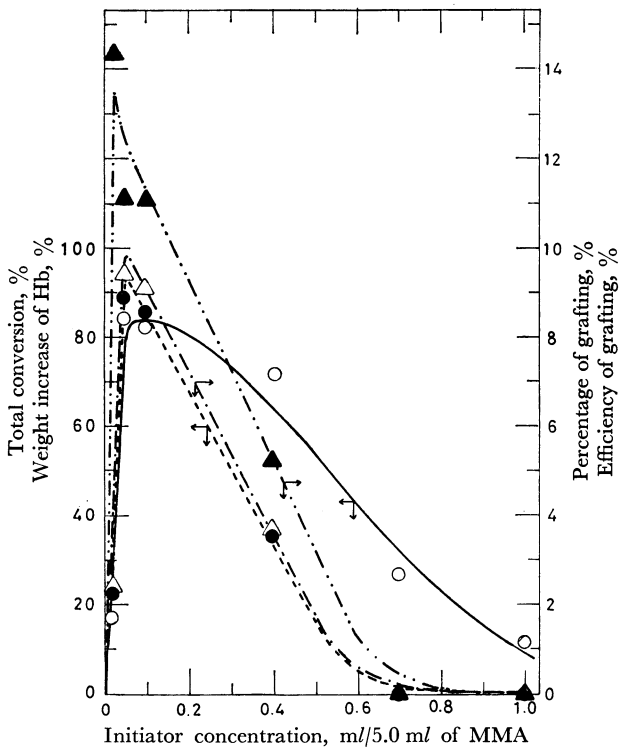

Fig. 5. Effects of initiator concentration.
Hemoglobin: 0.5 g; MMA: 5.0 m*l*; solvent: 10 m*l*; 37°C;
2 hr.

Concentration of Bu_3B. The dependence of the grafting on the concentration of Bu_3B is given in Fig. 5. There was an optimum concentration of 0.05—0.1 m*l* of Bu_3B/5.0 m*l* of MMA for the total conversion, the percentage of grafting, and the efficiency of grafting. All of these values decreased considerably when the concentration of Bu_3B increased much above the optimum concentration. A similar tendency was also observed in the system without hemoglobin.

Concentration of Hemoglobin. Figure 6 presents the relationship between the grafting and the concentration of hemoglobin. So long as the concentration was low, the total conversion was practically constant; when the former was higher than 0.4—0.5 g/5.0 m*l* of MMA, the latter decreased. Both the weight increase and the percentage of grafting had their optimum concentration of hemoglobin; especially, the weight increase gave relatively high values of 130—500% in the range of 0.05—0.30 g/5.0 m*l* of MMA. The higher the concentration of hemoglobin, the higher was efficiency of grafting.

On the Grafting Site. It is known that hemoglobin can accelerate the decomposition of hydrogen peroxide.

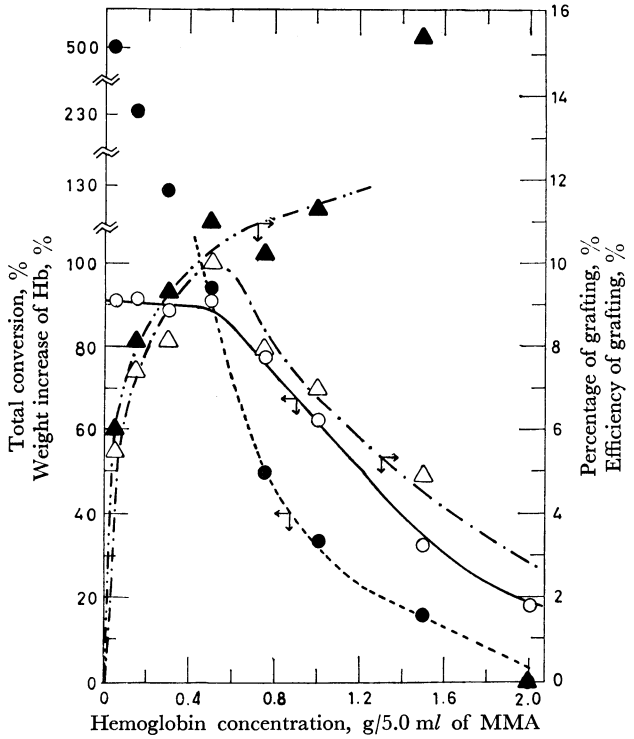

Fig. 6. Effects of homoglobin concentration.
(○): total conversion; (●): weight increase of homeglobin; (△): percentage of grafting; (▲): efficiency of grafting; MMA: 5.0 m*l*; Bu_3B: 0.10 m*l*; Solvent: 10 m*l*; 37°C; 2 hr.

This characteristic property remained practically unchanged after the graft copolymerization, as is shown in Fig. 7. This fact would suggest that MMA was not grafted onto the very part of the hemoglobin which possesses the hydrogen peroxide-decomposing ability. Fe^{2+} is known to be responsible for the decomposition of hydrogen peroxide. Therefore, Fe^{2+} may be supposed to be preserved without any change.

On the Reaction Mechanism. It is interesting to note again that the graft copolymerization of MMA by Bu_3B proceeded well only in aqueous media, although

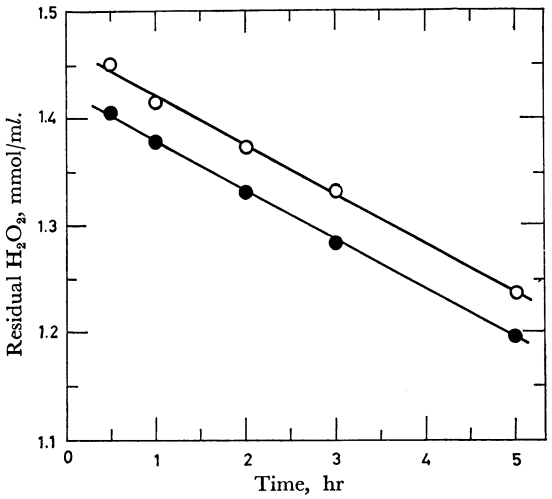

Fig. 7. Hydrogen peroxide-decomposing properties of hemoglobin and graft copolymer.
(○): hemoglobin; (●): graft copolymer. Initial concentration of H_2O_2: 1.46 mmol/m*l*; hemoglobin: 0.3 g; graft copolymer: 0.6 g.

water reacts with trialkylboranes to form hydroxy derivatives (*e.g.*, R_2BOH[18]) and, at last, inactive boric acid.[19] On the other hand, trialkylboranes can initiate the polymerization of MMA in ordinary organic solvents.[4-9] This dicrepancy could be explained by assuming the following three reaction steps:

Solvation:

$$Hb + n\cdot H_2O \longrightarrow (\text{solv. Hb}) \quad (1)$$

Formation of the complex:

$$(\text{solv. Hb}) + Bu_3B \longrightarrow \text{complex} \quad (2)$$

Formation of the active center:

$$\text{complex} \longrightarrow \text{active center} \quad (3)$$

to initiate the graft copolymerization

(Hb: hemoglobin)

Step 1 is supported by the fact that water is essential to the graft copolymerization and that no grafting occurred in the usual organic solvents. When proteins (such as albumin and casein) and fibers (such as wool, silk, and cotton) were treated similarly, the same phenomenon was also observed.[14]

We previously proposed the following reaction mechanism for the polymerization of MMA by the Bu_3B/pyridine system:[12]

$$\text{pyridine} + Bu_3B \longrightarrow \text{complex} \quad (4)$$

$$\text{complex} \longrightarrow \text{radical} \quad (5)$$

to initiate the polymerization

Steps 2 and 3 could be compared with Equations 4 and 5 respectively. The solvated hemoglobin seems to act as an electron donor in Step 2, just like pyridine in Eq. (4). All these backbone polymers contain hydrophilic groups such as amino and hydroxyl groups. The electron-donative property of the hydrophilic groups in hemoglobin is, therefore, believed to play an important role in the formation of the complex. Step 3 could be interpreted analogously in agreement with Eq. (5). In the Bu_3B/pyridine system, our ESR study suggested that the polymerization of MMA involves a free-radical mechanism.[12] Since (1) it is well known that the polymerization of vinyl monomers by trialkylboranes in the presence of oxygen or oxygen-containing compounds proceeds *via* a free-radical mechanism[6-8] and (2) radical polymerization is less sensitive to water than ionic polymerization, Step 3 may be supposed to involve a free-radical mechanism.

However, if an olefinic monomer is polymerizable by the free-radical mechanism, they can usually be polymerized with every other peroxide and azo initiator, too.[20] On the contrary, the benzoyl peroxide/dimethyl-*p*-toluidine system was ineffective on the graft copolymerization under the same conditions.

We thank Professor Eiichi Masuhara for his encouragement throughout this work. We are indebted to Mr. Akihiko Watanabe, Tokyo Medical and Dental University, for taking the electron micrograph.

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