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Infrared Spectroscopy of Graft Polymers Separated from Graft Copolymers of Wool and Silk with Methyl Methacrylate

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Synopsis

The HCl- or papain-decomposition residues obtained from reduced wool fibers grafted with 1.5–3.0% methyl methacrylate were extractively fractionated successively with cold acetone, hot acetone, and benzene. Each fraction was examined by infrared spectroscopy. Generally, the 715 cm^{-1} spectral band considered to be due to $\nu_{C\text{-}S\text{-}C}$ was found in the hot acetone-soluble fraction. It was concluded that the thiol groups on wool keratin provide the main sites of grafting, as might be expected. A similar result was obtained for grafted silk fibroin fibers, the methionine residue being presumed to be related to some of the grafting sites at least. Furthermore, a sort of stereoregularity was observed in some of the graft polymer fractions, isotactic-rich material being obtained in the case of silk fibers. In the range of very low grafting, a stereoregulating effect on the structure of the polymer formed within fibers appears to be present in relation to the grafting or adsorption sites of monomers and the fine structure of fibers.

INTRODUCTION

In our previous papers, it was reported that mainly the thiol groups on wool keratin could be considered to provide the sites of grafting in the free-radical graft copolymerization of vinyl monomers in wool fibers.[1–3]

Since protein materials contain various functional groups, studies on the grafting site appear to be difficult, while of interest. Up to the present, there have been few reports dealing primarily with this problem. Recently, some studies on the grafting of methyl methacrylate or acrylamide to ovalbumin[4] and acrylonitrile to collagen[5] have appeared.

In the present paper, we attempted to determine the grafting sites on the polypeptide chain of wool by the infrared spectroscopy of the graft polymers separated from the wool–methyl methacrylate graft copolymers, the protein fragment being left on the end of the graft polymers. To permit infrared spectroscopic determination, the relative concentration of endgroups on the graft polymers was increased, i.e., the studies were carried out on graft polymers with a relatively lower molecular weight. Therefore, on the basis of results obtained in the previous paper,[3] the grafting reaction was performed under the conditions of lower concentration of monomers and lower graft-on on strongly reduced wool fibers.

In addition, a similar experiment was carried out on the silk fibroin–methyl methacrylate graft copolymers.

EXPERIMENTAL

Materials

The wool fiber samples (Merino wool tops) were purified by extraction with acetone in a Soxhlet apparatus for about 24 hr, followed by washing with cold water and air drying. The silk fibroin fibers were obtained by scouring raw silk in an aqueous solution containing 0.4% soap for 2 hr and washing with 0.05% sodium carbonate solution, then with boiling water.

Methyl methacrylate (MMA) was purified as in the usual way. Potassium persulfate, thioglycolic acid (TGA), and sodium sulfide were of highest laboratory reagent quality and used without further purification, as were diethylene glycol monobutyl ether (BC), lithium bromide, and hydrochloric acid. Papain was a commercial product (Merck) having a digestive power of 1:350.

Reduction of Wool Fibers

The wool (1 g) was treated for 24 hr at 30°C with a 0.516*N* solution of TGA adjusted to pH 4.8 (100 ml), washed successively with water, ethanol, and water, pressed out with filter paper, and then subjected to the grafting. An alternative procedure was to treat the wool with 0.042*M* sodium sulfide solution for 20 min at 25°C at a liquor:wool ratio of 50:1. Thereafter the samples were washed in running water for 24 hr, treated with 0.01*N* hydrochloric acid for 24 hr, washed with distilled water, centrifuged, and conditioned at 65% RH for 3 days.

The reduced wool fibers with TGA and sodium sulfide were found to contain 31.0 and 21.7 mole thiol groups/10^5 g wool, respectively, from polarographic determination.[6]

Grafting Procedure

The graft copolymerization for the reduced wool was performed in the LiBr–$K_2S_2O_8$ system or with $K_2S_2O_8$ alone at 30°C for 3 or 15 min, following the procedure reported in the previous papers.[2,3] In the LiBr–$K_2S_2O_8$ system, the grafting proceeded with almost no homopolymer formation. Since the $K_2S_2O_8$ system without bromide allows some amount of homopolymer to form, the grafted wool fibers were Soxhlet-extracted with acetone for 24 hr and then washed with water. The grafting for the silk fibroin fibers was carried out in the LiBr–$K_2S_2O_8$ system at 40°C for 20 min with almost no homopolymer formation, and therefore acetone extraction was omitted. Also, the polymerization of methyl methacrylate homopolymer (atactic) was done in aqueous solution containing 0.2% $K_2S_2O_8$, 2% MMA, and 22.5% BC at 30°C for 3 hr.

Separation and Fractionation of Graft Polymers

In the previous study,[3,8] in order to separate the MMA graft polymers from the wool or silk trunks, the digestion of the graft copolymers was done in 6*N* HCl for 24 hr at 115°C. This procedure is suitable for measurement of the molecular weight of graft polymers. By adopting more mild conditions for decomposition of wool trunks, the protein fragment can be retained attached to the end of the graft polymer. We therefore carried out the decomposition in 6*N* HCl for 24 hr at 60°C at a liquor ratio of 70:1. The same procedure of decomposition was also tried for the MMA–silk graft copolymers.

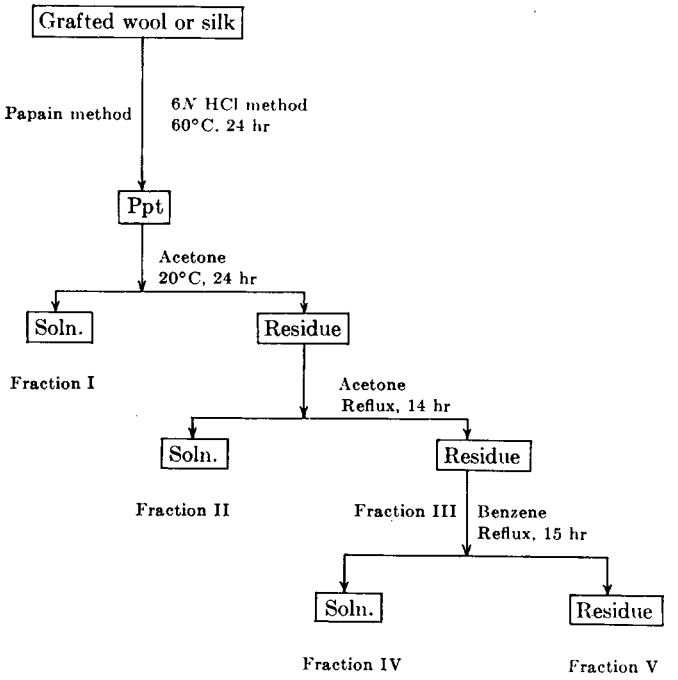

Fig. 1. Fractionation scheme.

Also, enzyme decomposition with papain, which was considered to bring about a milder decomposition of wool, was carried out according to the procedure of Lennox,[7] the grafted wool fibers being treated in aqueous solutions containing 0.1*M* sodium bisulfite, 3*M* urea, and 3% papain for 24 hr at 55°C, filtered, washed with water, and dried.

The products separated from the wool or silk trunks were fractionated into five fractions according to the scheme shown in Figure 1.

The purpose of fractionation is to prepare samples graded with respect to the extent to which the poly(methyl methacrylate) chain or the protein fragment attached to the chain end contributes to the solubility. Fraction I, the easily acetone-extractable part (20°C, 24 hr), is the material in which the

contribution of the protein fragment on the end of MMA polymer should be least. Naturally, this fraction may contain some amount of MMA homopolymer formed within the fibers. Successively, the unextractable residues were refluxed with the hot acetone for 14 hr with the use of the Soxhlet apparatus. The soluble part is fraction II. This fraction should contain the MMA polymer in which the contribution of the protein fragment is moderately great. Fraction III, the insoluble residue from this extraction, was again refluxed for 15 hr with hot benzene which is considered to be a somewhat better solvent for poly(methyl methacrylate). Thus the extracted part, fraction IV, will have a quite high concentration of protein fragments. The benzene-insoluble part, fraction V will be composed of relatively long polypeptides with or without a short chain of MMA polymer on the end.

Color Identification Tests of Fractions

As with the fractions obtained above, some tests of color identification on protein fragment were carried out. Determinations by the ninhydrin reaction, for the presence of amino acid residues or polypeptide having free carboxyl and amino groups, Millon's reaction for tyrosine residues, and by Feigl's method[9] for bivalent sulfur for cystine, cystein, or methionine residues were carried out.

Infrared Spectroscopic Analysis

The infrared spectra of each fraction of the decomposition residues obtained from grafted wool or silk, the atactic poly(methyl methacrylate)

TABLE I
MMA Graft-on and Extent of Decomposition of Polypeptide Portion

Sample used for grafting	Grafting system	Graft-on, %	Decomposition agent of polypeptide	Extent of decomposition of polypeptide, %
Untreated wool	—	—	HCl	99.8
Untreated wool	—	—	Papain	98.5
TGA-reduced wool	LiBr–$K_2S_2O_8$[a]	3.03	HCl	97.6
Na_2S-reduced wool	LiBr–$K_2S_2O_8$[a]	2.51	HCl	98.0
TGA-reduced wool	$K_2S_2O_8$[b]	2.92	HCl	97.6
Na_2S-reduced wool	$K_2S_2O_8$[b]	2.91	HCl	97.7
TGA-reduced wool	LiBr–$K_2S_2O_8$[c]	1.58	Papain	96.6
Silk fibroin	LiBr–$K_2S_2O_8$[d]	1.50	HCl	88.5

[a] LiBr, 27.5%; $K_2S_2O_8$, 0.2%; MMA, 1.0%; H_2O, 48.8%; BC, 22.5% (by weight); 30°C; 15 min.

[b] $K_2S_2O_8$, 0.2%; MMA, 1.0%; H_2O, 76.3%; BC, 22.5% (by weight); 30°C; 15 min.

[c] LiBr, 27.5%; $K_2S_2O_8$, 0.2%; MMA, 2.0%; H_2O, 47.8%; BC, 22.5% (by weight); 30°C, 3 min.

[d] LiBr, 25.0%; $K_2S_2O_8$, 0.2%; MMA, 2.0%; H_2O, 50.3%; BC, 22.5% (by weight); 40°C; 20 min.

homopolymer, and the decomposition product of untreated wool were measured with a Nippon Spectroscopic DS-301 spectrophotometer in the form of KBr pellets (500 mg) containing 2–6 mg of powdered polymers.

RESULTS AND DISCUSSION

Extent of Decomposition of Polypeptides

The graft-on in MMA-grafted wool or silk and the extent of decomposition of the polypeptide portion by hydrochloric acid or papain are shown in Table I.

The extent of decomposition of polypeptide part in the wool grafted with 1.5–3.0% MMA amounted to 97–98% in both HCl and papain decomposition, being slightly less than that in the untreated wool. These results indicate that the isolated graft polymers contain roughly equivalent weights of fragments of polypeptide. The extent of HCl decomposition of the grafted silk was somewhat less than that in the grafted wool.

Yield in Solvent-Extractive Fractionation

The yields in the solvent-extractive fractionation of the residues obtained by decomposing the graft copolymers with HCl or papain are shown in Table II.

The total yield of each fraction amounted to 70–100% in all samples.

Color Reactions of Fractions

Table III shows the results of the color identification tests of the solvent-extracted fractions from the residue obtained by HCl or papain decomposition of the MMA–wool graft copolymers. Bivalent sulfur and ninhydrin reactions were positive in all fractions, revealing the presence of protein fragments. Millon's reagent gave a positive reaction only in fractions III and V, in which the content of protein fragments is considered to be relatively high. Also, the bivalent sulfur reaction is relatively strong in fraction III.

These results indicate that the fractionation has been carried out smoothly.

Infrared Spectra of Fractions

Figure 2, illustrating the infrared spectrum of HCl decomposition residue of the ungrafted wool shows characteristic amide absorption bands at 1550 (amide II) and 1660 ($\nu_{C=O}$) cm^{-1}. The absorption bands of the atactic poly(methyl methacrylate) homopolymer appear at 750 (γ_{CH_2}), 1730 ($\nu_{C=O}$), and near 3000 cm^{-1} (ν_{sCH_3O}, ν_{aCH_3O}) as shown in Figure 2.

As with the HCl decomposition residue obtained from the 3.03% MMA-grafted wool, the spectra of fractions I, II, and III are shown in Figure 3. The spectra of fractions I and II indicate almost the same bands as for the poly(methyl methacrylate) homopolymer, although the presence of amide absorption (1550 and 1660 cm^{-1}) appears faintly. The spectrum of frac-

TABLE II
Yield in Solvent-Extractive Fractionation of Residue Obtained by Decomposition of Polypeptide Portion

Sample used for grafting	Graft-on, %	Decomposition agent of polypeptide	Weight of decomposition residue obtained, g	Yield of each fraction, %					Total yield, %	
				I	II	III	IV	V	Fractions I, II, and III	Fractions I, II, IV, and V
TGA-reduced wool	3.03	HCl	0.0612	37.1	12.6	45.9	—	—	95.6	—
Na_2S-reduced wool	2.51	HCl	0.0475	22.3	11.1	34.8	—	—	68.2	—
TGA-reduced wool	2.92	HCl	0.0561	33.8	20.3	45.1	—	—	99.2	—
Na_2S-reduced wool	2.91	HCl	0.0325	30.2	20.6	30.2	—	—	81.0	—
TGA-reduced wool	1.58	Papain	0.0830	23.8[a]	2.2	53.5	3.0	40.0	79.5	69.0
Silk fibroin	1.50	HCl	0.0648	4.1	6.9	89.5	—	—	100.5	—

[a] Soluble in acetone, 50°C.

TABLE III
Color Identification Tests of Solvent-Extracted Fractions from Decomposition Residue of MMA–Wool Graft Copolymers

Sample	Graft-on, %	Decomposition agent of wool part	Fraction	Color reaction			Infrared absorption band at 715 cm^{-1}
				Bivalent sulfur	Ninhydrin	Millon's	
Untreated wool	—	HCl	—	++	+	+	−
TGA-reduced wool	3.03	HCl	I		+	−	−
			II	+	+	−	+
			III	++	+	+	−
Na_2S-reduced wool	2.51	HCl	I	+	+	−	−
			II	+	+	−	+
			III	++	+	+	−
TGA-reduced wool	2.92	HCl	I	+			−
			II	+			+
			III	++			−
Na_2S-reduced wool	2.91	HCl	I	+			−
			II	+			+
			III	++			−
TGA-reduced wool	1.58	Papain	I		+	−	+
			II		+		+
			IV		+	−	+
			V		+	+	−
MMA homopolymer	—	—	—	−	−	−	−

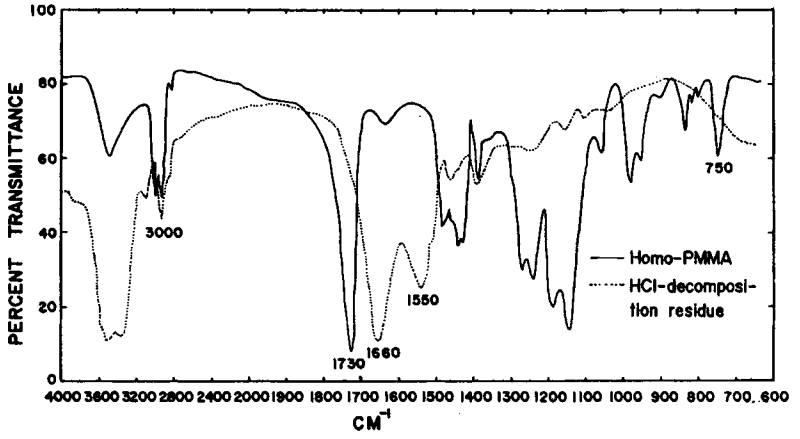

Fig. 2. Infrared spectra of the HCl-decomposition residue of ungrafted wool and atactic poly(methyl methacrylate) homopolymer.

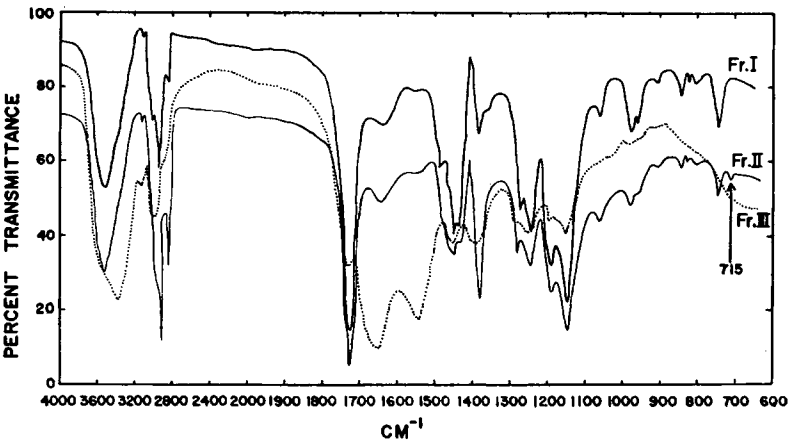

Fig. 3. Infrared spectra of fractions of HCl-decomposition residue obtained from 3.03% MMA-grafted wool.

tion III is very similar over the whole spectral range to that of the decomposition product of untreated wool, the absorption band of the ester group of poly(methyl methacrylate) (1730 cm^{-1}) being weakened markedly. It is noteworthy that the absorption peak at 715 cm^{-1} which is not present in the decomposition product of wool appears weakly yet characteristically in fraction II.

Figure 4 shows the infrared spectra measured on fractions I, II, IV, and V of the papain-decomposition residue obtained from the wool grafted with 1.58% MMA. Fractions I, II, and IV exhibit the characteristic absorption bands of poly(methyl methacrylate) (750 and 1730 cm^{-1}) strongly and that of the amide group (1550 and 1660 cm^{-1}) weakly. On the other hand, fraction V exhibits strong amide absorptions and weak poly(methyl meth-

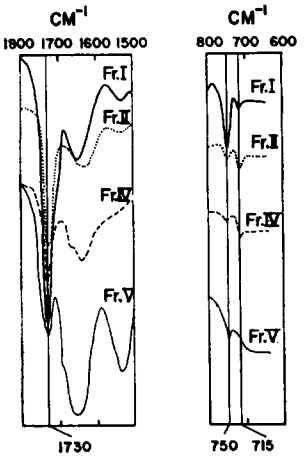

Fig. 4. Infrared spectra of fractions of papain-decomposition residue obtained from 1.58% MMA-grafted wool.

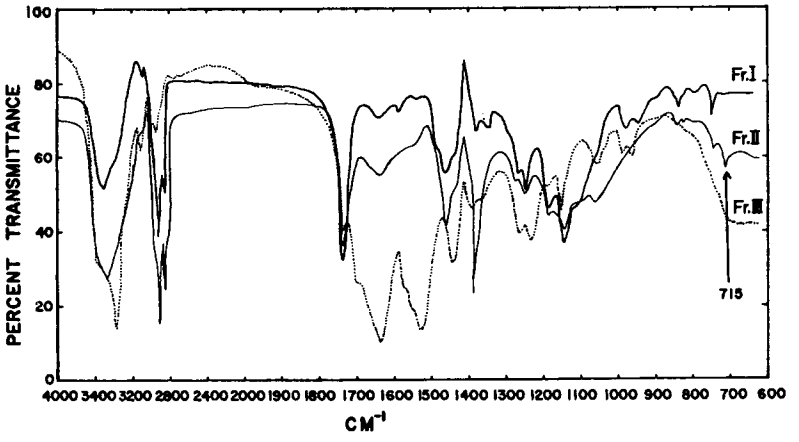

Fig. 5. Infrared spectra of fractions of HCl-decomposition residue obtained from 1.50% MMA-grafted silk fibroin.

acrylate) absorptions, showing it to be most rich in protein fragments. Here again, it is worthy of note that fractions I, II and IV exhibit the weak but fairly obvious peak at 715 cm^{-1} which is not present in either fraction V or papain itself.

As shown in the results of infrared analysis carried out for each fraction of other samples, generally the 715 cm^{-1} spectral band appears in the hot acetone-soluble fraction (see Table III). There is a possibility that the particular absorption band at 715 cm^{-1} is due to the amino acid residue as the grafting site which is attached directly to the end of graft polymer. To clarify whether this spectral band is due to a fragment of incompletely decomposed polypeptide, fraction II obtained from the HCl-decomposition

product of the wool grafted with 2.91% MMA was again treated with 6*N* HCl for 24 hr at 110°C and subjected to the infrared analysis. The result revealed that the 715 cm^{-1} spectral band did not change on retreatment with hydrochloric acid.

As reported in our previous papers,[1-3] if the thiol groups on wool keratin are the main sites of grafting, the absorption spectrum due to the C—S—C bond should appear. It is known that the absorption band of ν_{C-S-C} is present in the range of 600–750 cm^{-1} and the intensity is weak generally.[10,11] The absorption of —S—S— bond is present in the range of lower frequency (400–500 cm^{-1}).[12] As seen in Table III, the protein-rich fractions give a stronger color reaction for bivalent sulfur. This may perhaps be due to the —S—S— bond in the cystine residue. From the above results, we conclude that the spectral band at 715 cm^{-1} is due to the C—S—C bond and indicative of the grafting site expected.

The spectra of the fractionation products obtained from the HCl-decomposition product of grafted silk fibroin are shown in Figure 5, indicating a similar result to that for wool. Here interestingly, the 715 cm^{-1} spectral band appears also in fraction II. We have reported that the kinetics of graft copolymerization in silk fibroin are clearly different from that of wool keratin, the apparent energy of activation in graft copolymerization being 10.1 kcal/mol g in silk and 4.2 kcal/mol g in wool.[8] Recently, Needles has reported that the cystine, tyrosine, and methionine residue on wool or silk are especially easily attacked with persulfate.[13,14] Considering that the tyrosine residue does not participate in the graft copolymerization of silk,[2] and the silk fibroin does not contain cystine but minute amounts of methionine residue, it is presumed that the 715 cm^{-1} spectral band is due to the methionine residue for part of the grafting sites at least. This point is now being investigated in detail.

Structure of Graft Polymer

From the viewpoint of the structure of poly(methyl methacrylate), it is noted that the infrared spectra of fractions obtained from the HCl- or papain-decomposition product of grafted wool or silk are different from the spectrum of the atactic poly(methyl methacrylate) homopolymer (see Figs. 2–5). Changes of absorption bands are noted especially in the ranges of 2800–3000 cm^{-1} and 1400–1500 cm^{-1}. In Figure 6 illustrating the spectra of fractions obtained from the papain-decomposition product of 1.58% MMA-grafted wool, the changes of absorption band in both ranges of frequency are shown in detail. In fraction I, the spectrum is on the whole similar to that of the atactic homopolymer, while in fractions II and IV, the peaks at 1483, 2940, and 2995 cm^{-1} decay to shoulders, and the weak peak or shoulder bands at 1465, 2840, and 2920 cm^{-1} increase distinctly.

Furthermore, the peak at 1063 cm^{-1} appears in both atactic homopolymer and most of graft polymer fractions (fractions I and II) separated from the decomposition product of grafted wool or silk, while it does not in the fraction I separated from the decomposition product of grafted silk fibroin,

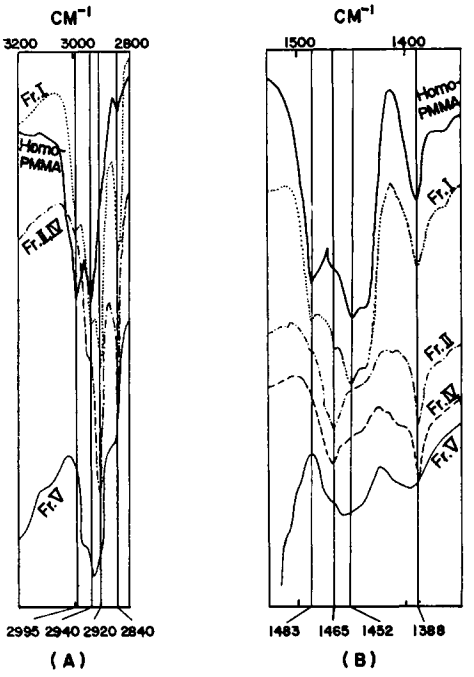

Fig. 6. Infrared spectra of fractions of papain-decomposition residue obtained from 1.58% MMA-grafted wool: (*A*) 2800–3000 cm^{-1}; (*B*) 1400–1500 cm^{-1}.

as shown in Figure 5. The molecular weight of this fraction was determined to be 52,000, giving a greater value than that of the graft polymer isolated from 3.03% MMA-grafted wool (18,000).

Considering the differences in the absorption bands at 1483 and 1063 cm^{-1} which are absent for pure isotactic poly(methyl methacrylate),[15,16] the structure of poly(methyl methacrylate) in the fraction I separated from the grafted silk fibroin is considered to be rich in isotactic polymer. This result suggests formation of some sort of stereoregular poly(methyl methacrylate) to some extent in the wool fibers, and especially the silk fibroin fibers. This result is very interesting, as it seems to indicate a stereoregulating effect on the structure of polymer formed within fibers in relation to the grafting or adsorption site of monomers and the fine structure of fibers. Some of the fractions mentioned above have been verified to be crystalline to some extent by x-ray diffraction method. This will be discussed in detail in a future report.

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