Amylose-Carrying Styrene Macromonomer and Its Homo- and Copolymers: Synthesis via Enzyme-Catalyzed Polymerization and Complex Formation with Iodine

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ABSTRACT: The objective of our research was to prepare novel conjugates between polysaccharides and vinyl polymers by applying enzymes as polymerization catalyst for polysaccharide synthesis. An approach was attained using amylose-substituted styrene macromonomers (vinylbenzyl amylose amide, VAA; the number-average degree of polymerization of amylose = 24 and 150) which were synthesized from maltopentaose-substituted styrene (VM5A) by phosphorylase-catalyzed polymerization of glucose 1-phosphate. Radical homo- and copolymerization of VAA gave an uncommon type of graft copolymers consisting of polystyrene and polyacrylamide backbones and amylose side chains of uniform length. Water-insoluble amylose was solubilized into water by incorporating VAA units into polyacrylamide main chains and also by hydroxypropylation of the amylose moieties of water-insoluble copolymers. Structural features of these graft copolymers were discussed on the basis of amylose−iodine complexation investigated by UV spectroscopy. Schematic structures of two different types of polystyrene-*graft*-amylose prepared via homopolymerization of VAA and via enzymatic elongation from poly(VM5A) were proposed. These amylose-carrying polyacrylamide and polystyrene prepared by applying enzyme-catalyzed polymerization are of interest as a new type of biomedical material and a well-defined model for conformational analysis.

1. Introduction

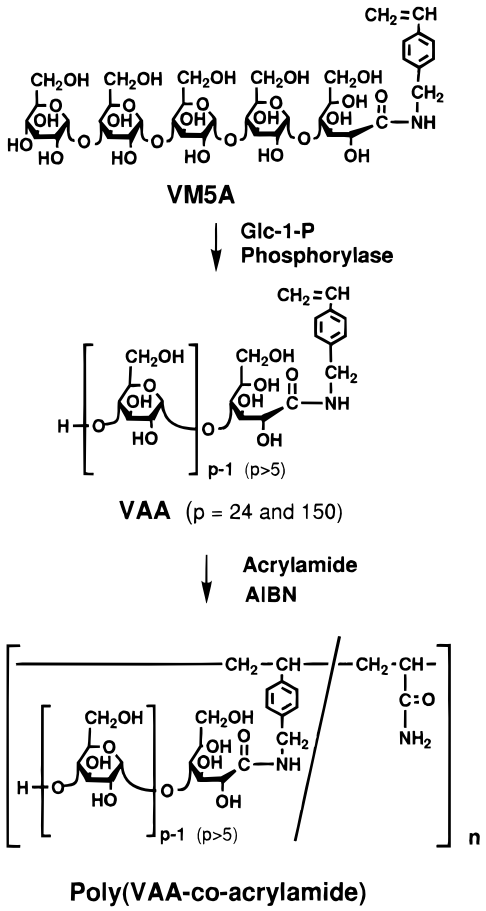
Application of enzymatic catalysts has been important in synthetic polymer chemistry from the aspects of development of biodegradable polymers[1,2] as well as effective utilization and recycling of natural resources. This paper reports the synthesis of graft copolymers with amylose side chains via phosphorylase-catalyzed synthesis of amylose-substituted styrene macromonomer (vinylbenzyl amylose amide, VAA) according to Scheme 1. An amylose chain with a rather uniform degree of polymerization is connected to a vinylbenzyl group via a gluconamide linkage in VAA macromonomer. Homopolymerization of the macromonomer and its copolymerization with acrylamide gave polystyrene-*graft*-amylose and poly(VAA-*co*-acrylamide).

Graft copolymers consisting of polysaccharide backbones and synthetic polymer grafts are quite common. However, little is known about graft copolymers consisting of synthetic polymer backbone and polysaccharide grafts, except the enzymatic grafting of amylose onto synthetic polymers, which has been pioneered by Pfannemüller.[3−6] Amylose-iodine complexation of the present graft copolymers was investigated by UV spectroscopy and discussed in connection with structural features of different type of amylose-carrying homo- and copolymers.

2. Experimental Section

Potato α-glucan phosphorylase (EC 2.4.1.1) was prepared and purified by the method of Kamogawa et al.[7] Crude extract from 10 kg of potato contained 57.4 g of protein, its total activity determined by the liberated phosphate was 27.5×10^3 units, and the specific activity was 0.48 units/mg. After

Scheme 1. Synthesis of Amylose-Carrying Styrene Macromonomers and Their Radical Homo- and Copolymerizations with Acrylamide



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purification, the product contained 270 mg of protein, its total activity was 6011 units, and hence the specific activity was 22.3 units/mg. Acrylamide and 2,2′-azobisisobutyronitrile were recrystallized, respectively, from ethyl acetate solution and from ethanol solution. Dimethyl sulfoxide was purified by distillation under reduced pressure.

NMR spectra were recorded on a JEOL JNM-FX-270 spectroscope operating for ^{1}H-NMR at 270 MHz (pulse width 5.0 μs, scan times 16, and relaxation delay 137.9 μs) and for ^{13}C-NMR at 67.80 MHz (pulse width 3.9 μs, scan times 1.0×10^4, and relaxation delay 24.0 μs). Absorption spectra were taken on a JASCO Ubest-30 UV/vis spectrophotometer. Size exclusion chromatography (SEC) was recorded with a Shimadzu HPLC on Toso-gel G-3000PWXL and G-2500PWXL columns for VAA [number-average degree of polymerization (DP_n) = 24] (0.5 M KCl aqueous solution) and on Toso-gel G-3000PWXL and G-6000PWXL columns for VAA (DP_n = 150 and 450) (0.25 M KCl aqueous solution) and with a JASCO LC800 System on Shodex B804 → 805 columns for copolymers (H_2O). RI detector and UV detector (wavelength, 280 nm) were connected in series.

Monodisperse amylose samples (Nakano Vinegar Co. Ltd., Handa, Aichi, Japan), maltopentadecaose (G15, Nakano Vinegar), maltopentaose (Hayashibara, Okayama, Japan), and maltose (Hayashibara) were used as the molecular weight standards. The monodisperse amyloses ($M_w = 5.62 \times 10^3$ to 1.7×10^6) were prepared via enzyme-catalyzed synthesis from maltopentaose and characterized on the basis of sedimentation equilibrium and light scattering measurements in Me_2SO by Nakanishi et al.[8]

***N*-(*p*-Vinylbenzyl)[*O*-α-D-glucopyranosyl-(1→4)-]$_4$-D-gluconamide (Vinylbenzyl Maltopentaose Amide, VM5A).**[9] Maltopentaose (15 g) was dissolved in a small amount of distilled water. An iodine (9.3 g) solution in methanol (70 mL) was added to the solution, and then 4% potassium hydroxide in methanol solution (240 mL) was added dropwise at 40 °C for 15 min. The mixture was poured into ice−water. Precipitated product was filtered and dissolved into distilled water (200 mL); the solution was treated with activated carbon and filtered; the filtrate was freeze-dried. The product was dissolved in distilled water, treated with Amberlite IR-120 (H^+), concentrated in a rotary evaporator, and then freeze-dried. The yield of maltopentaose lactone was 13.3 g.

The lactone (13.3 g) was dissolved in ethylene glycol (70 mL). To the solution was added *p*-vinylbenzylamine (2.2 g) solution in ethylene glycol (15 mL) and the mixture was heated at 70 °C for 6 h. The product was precipitated in acetone (1.5 L), washed with acetone (800 mL), and dried under reduced pressure at 60 °C for 3 h. The yield was 10.1 g.

To remove unreactive oligosaccharide derivatives, the crude product (10 g) was purified with a Waters preparative high performance chromatography system equipped with a YMC ODS column (S-5 120A AQ, 20 $\phi \times$ 250 cm) and a RI detector using a mixture of water and acetonitrile (78:22) as eluent. Flow rate was 6 mL/min.

***N*-(*p*-Vinylbenzyl)[*O*-α-D-glucopyranosyl-(1→4)]$_{(p-1)}$-D-gluconamide (VAA, *p* = 24).** VM5A (1.08 g, 1.09 mmol) and potassium α-D-glucose-1-phosphate dihydrate (14 g, 38 mmol) were dissolved in a mixture of 0.1 M maleic acid buffer (pH = 7.5, 363 mL) and dimethyl sulfoxide (120 mL), and the mixture was heated to 45 °C. Potato α-glucan phosphorylase (80 units) was added and the solution was incubated at 45 °C for 10 h. Liberated phosphate was determined according to the method of Fiske and Subbarow.[10] The solution was heated at 95 °C for 5 min to deactivate the enzyme and then immediately cooled to 40 °C. The precipitated protein was removed by filtration. The filtrate was poured into ethanol (500 mL) and the supernatant was removed by centrifugation. The precipitate was washed with ethanol and diethyl ether and dried *in vacuo* at 70 °C. White powdery VAA macromonomer was isolated in a 2.0 g yield.

In SEC, two peaks appeared at R_f = 20.6 and 23.4 min by reflective index (RI) detection, while only one peak appeared at R_f = 23.3 min by UV detection at 280 nm. Since the VAA macromonomer was susceptible to both detectors, the undetectable peak by UV at R_f = 20.6 min was attributable to the amylose having no vinylbenzyl moiety. According to the peak area, 16 wt % of amylose contaminated the VAA. The number-average polymerization of amylose chains (DP_n = 24) in VAA was determined by assuming that VAA macromonomer has the same DP_n as the amylose having no vinylbenzyl group. Purification by HPLC of a small scale sample was attained, but an unpurified sample was used for the following experiments.

^{1}H NMR (Me_2SO-d_6, 5.0%, 70 °C): δ 7.94 (t, J = 6.0 Hz, NH); 7.32 (m, J = 10.2 Hz, phenyl); 6.71 (dd, J = 10.8 and 17.9 Hz, C*H*=CH_2); 5.75 (d, J = 17.9 Hz, CH=CH_2 (cis)); 5.18 (s, OH-2, OH-3, CH=CH_2 (trans)); 5.10 (s, H-1); 4.31 (s, OH-6); 3.63 (m, H-3, H-5, H-6a, H-6b); 3.36 ppm (m, H-2, H-4). ^{13}C NMR (Me_2SO-d_6, 5.0%, 70 °C): δ 139.1, 136.3, 127.2, and 125.8 (phenyl); 136.9, and 113.1 (CH=CH_2); 99.9 (C-1); 78.7 (C-4); 73.1 (C-3); 71.9 (C-2); 71.5 (C-5); 62.5 (C-6 of the reducing terminal gluconamide unit); 60.4 ppm (C-6).

***N*-(*p*-Vinylbenzyl)[*O*-α-D-glucopyranosyl-(1→4)]$_{(p-1)}$-D-gluconamide (VAA, *p* = 150).** VM5A (0.041 g, 0.043 mmol) and potassium α-D-glucose-1-phosphate dihydrate (4 g, 11 mmol) were dissolved in a mixture of 0.1 M maleic acid buffer (pH = 7.5, 100 mL) and dimethyl sulfoxide (35 mL) and heated to 45 °C. Potato α-glucan phosphorylase (45 units) was added and the solution was incubated at 45 °C for 18 h. The post-treatment was described above. White powdery VAA macromonomer was isolated in a 0.95 g yield.

Homopolymerization of VAA Macromonomers and Their Copolymerization with Acrylamide. A representative procedure is as follows. A solution of 2,2′-azobisisobutyronitrile (23 mg/5 mL) in dimethyl sulfoxide was prepared. VAA macromonomer (DP_n = 24, 100 mg) and acrylamide (400 mg) were dissolved in a mixture of the initiator solution (1 mL) and dimethyl sulfoxide (0.5 mL) in an ampule. The solution was diluted with water (1.5 mL), cooled to −78 °C, and deaerated *in vacuo*. The ampule was sealed *in vacuo* and kept at 60 °C in a thermostated water bath for 3 h. The solution was poured into methanol, the supernatant was removed by decantation, and the precipitated product was dried *in vacuo*.

The copolymers of VAA macromonomer (DP_n = 24) was isolated as follows. The product was immersed in cold water. The cold-water-soluble fraction (acrylamide-rich copolymer, fraction I) was separated by centrifugation and freeze-dried. The cold-water-insoluble fraction was heated in water at 98 °C for 1 h and centrifuged (20 °C) to separate the hot-water-soluble fraction (fraction II) and insoluble fraction (fraction, III). Fraction II was freeze-dried and fraction III (VAA-rich copolymer and/or homopolymer of VAA) was dried *in vacuo*.

The following are the NMR data of the VAA homopolymer (DP_n of amylose = 24). ^{1}H-NMR (Me_2SO-d_6, 1.7%, 27 °C): δ 5.48 (s, OH-2); 5.38 (s, OH-3); 5.11 (s, H-1); 4.88 (s, the hydroxyl of terminal glucose unit), 4.56 (s, OH-6); 3.62 (m, H-3, H-5, H-6a, H-6b); 3.36 ppm (m, H-2, H-4). ^{13}C-NMR (Me_2SO-d_6, 1.7%, 70 °C): δ 99.7 (C-1); 78.6 (C-4); 72.9 (C-3); 71.8 (C-2); 71.4 (C-5); 60.3 ppm (C-6).

The composition of VAA in copolymers was estimated from the ^{1}H-NMR area ratio of the main chain methylene and methyne signals (δ 2.2 and 1.5 ppm) and the glucopyranose signals.

Acrylamide-rich copolymers of VAA macromonomer (DP_n = 40 and 150) were isolated as cold-water-soluble products.

Enzyme-Catalyzed Polymerization of Maltopentaose-Carrying Polystyrene (PolyVM5A) as Primer. Radical homopolymerization of VM5A (0.96 g, 1 mmol) was carried out with potassium peroxodisulfate (0.2 mol %) as initiator in water (1.2 mL) at 60 °C for 11 h.[9] Polymer yield, 69%; $[\alpha]^{25}_D$ = +146° (c = 1 g/100 mL).

Polystyrene-*graft*-amylose (DP_n = 50) was synthesized via enzyme-catalyzed polymerization of polyVM5A with glucose 1-phosphate using potato α-glucan phosphorylase in 0.1 M maleic acid buffer containing dimethyl sulfoxide (20−30%). The polymer was soluble in dimethyl sulfoxide and insoluble in hot water.

Preparation of Hydroxypropylated Copolymers.[11] The copolymer (expt no. 33, fraction III, DP_n of amylose = 24, wt % of VAA = 93, 20 mg) was dissolved in dimethyl sulfoxide

Table 1. Copolymerization of VAA Macromonomer (DP_n of Amylose = 24) with Acrylamide (60 °C)

expt no.	VAA,[a] mg	acrylamide, mg	VAA in monomer mol %	VAA in monomer wt %	AIBN,[b] mol %	Me_2SO, mL	H_2O, mL	time, h	yield fr I,[c] %	yield fr II,[d] %	yield fr III,[e] %	VAA in copolymer[f] fr I mol %	VAA in copolymer[f] fr I wt %	VAA in copolymer[f] fr III mol %	VAA in copolymer[f] fr III wt %
21	0	1422	0	0	0.5	5.0		1	84	0	0	0	0		
27	75	709	0.2	10	0.5	5.0	5.0	3	95[h]	0	0	0.2	10		
45	100	400	0.4	20	0.5	1.5	1.5	3	96[i]	3	0	0.3	16		
26	75	213	0.6	26	0.5	1.5	1.5	3	85[j]	0	0	0.8	32		
25[g]	113	213	0.9	35	0.5	2.0	2.0	1.5	14	24	1	3.6	68		
31	95	41	4.0	70	0.5	0.5	0.3	13	10	8	4	6.3	79	6.4	80
33	200	34	9.5	85	0.9	0.5	0.2	24	5	21	51	13	89	19	93
34	100	0	100	100	12	0.5		24	0	27	18			100	100
38	100	0	100	100	4.9	0.5		15	0	76	20			100	100

[a] Containing 16 wt % of amylose. [b] 2,2′-Azobisisobutyronitrile. [c] Soluble in cold water. [d] Soluble in hot water (98 °C). [e] Insoluble in hot water (98 °C). [f] Determined by ^{1}H-NMR. [g] 54−60 °C. [h] $M_n = 2.6 \times 10^5$. [i] $M_n = 2.0 \times 10^5$. [j] $M_n = 1.8 \times 10^5$, M_n was determined by SEC, with poly(ethylene oxide) standard in water.

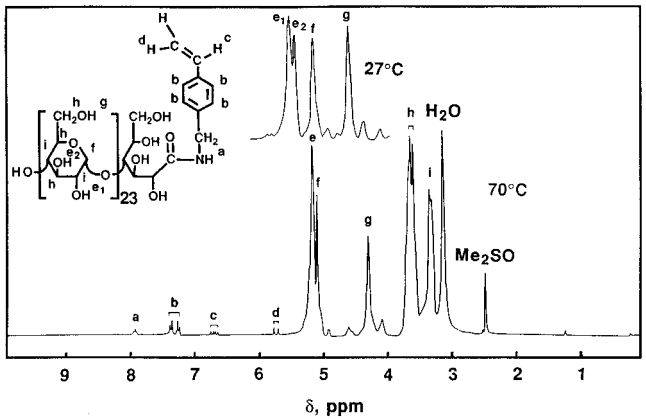


Figure 1. ^{1}H-NMR spectrum of VAA macromonomer (*N*-(*p*-vinylbenzyl)[*O*-α-D-glucopyranosyl-(1→4)]$_{23}$-D-gluconamide): concn, 5% in Me_2SO-d_6; temp, 27 and 70 °C; reference, TMS; 270 MHz.

(0.3 mL), and 0.2 M NaOH (1 mL) solution and then methyloxirane (0.2 mL) were added. The solution was stirred for 15 min and kept at room temperature for 2 h. The solution was diluted with water (10 mL), neutralized with 0.2 M HCl, and then dialyzed in a cellulose tube (Nacalai tesque; cutoff MW, 3500; diameter, 11 mm; thickness, 0.03 mm). The resulting polymer was freeze-dried and obtained as a white powder (29 mg).

Complex Formation of Iodine with Amylose in VAA and Copolymers. A standard iodine−iodide solution was prepared by dissolving potassium iodide (0.52 g) and iodine (0.52 g) in water (1 L). Amylose derivative (1.0 mg) was dissolved in dimethyl sulfoxide (0.2 mL) in a 10 mL volumetric flask, an aliquot (1 mL) of the standard solution was added, the resulting solution was diluted with water to 10 mL, and the absorption spectrum was taken.

3. Results and Discussion

3.1. Amylose-Carrying Styrene Macromonomer (VAA). VAA was synthesized via enzyme-catalyzed polymerization of glucose 1-phosphate onto VM5A as primer in a mixture of maleic acid buffer and dimethyl sulfoxide. The addition of dimethyl sulfoxide was effective to increase the solubility of the resulting amylose macromonomer and to avoid its deposition during the reaction.

When the DP_n of amylose was not large, the vinyl benzyl and amylose moieties were detected in ^{1}H- and ^{13}C-NMR spectra, the former of which is shown in Figure 1. The chemical shifts of hydroxyl protons (e and g) were dependent on the temperature, as discussed in the later section. The DP_n of amylose chains was 24 ($M_n = 4.0 \times 10^3$), which was determined as the average value of the following three methods: (a) the area ratio of the amylose signals to the vinyl benzyl signals in the ^{1}H-NMR spectrum (DP_n = 28), (b) the quantitative analysis[10] of phosphoric acid liberated during the reaction (DP_n = 20), and (c) size exclusion chromatography (SEC) (DP_n = 24, M_w/M_n = 1.20). The amylose-carrying macromonomer (VAA, DP_n = 24) was soluble in dimethyl sulfoxide and hot water (98 °C) and insoluble in cold water.

VAA macromonomer (DP_n of amylose = 150) isolated was soluble in dimethyl sulfoxide, but insoluble in hot water (98 °C). The vinylbenzyl signals in this macromonomer were not detectable in NMR spectra, and hence the DP_n was determined to be 150 (Mn = 2.4×10^4) as the average of the values estimated by phosphoric acid analysis (DP_n = 143) and SEC analysis (DP_n = 155, M_w/M_n = 1.14).

VAA macromonomer (DP_n of amylose = 40) was deposited during the reaction. The isolated product was insoluble in hot water and partially soluble in dimethyl sulfoxide. Amyloses having this range of DP_n, especially, are highly crystalline, so that the lack of solubility and the broadening of molecular weight distribution (M_w/M_n estimated by SEC = 1.41) were induced.

3.2. Polyacrylamide Having Amylose Chains as Graft Component, Poly(VAA-*co*-acrylamide). (a) Copolymerization of VAA Macromonomers with Acrylamide. Homo- and copolymerizations of VAA macromonomer (DP_n of amylose = 24) with acrylamide were carried out using 2,2′-azobisisobutyronitrile as initiator at 60 °C as summarized in Table 1. Mixtures between dimethyl sulfoxide and water were used as the polymerization solvents. The products were fractionated into three fractions on the basis of the solubility. Acrylamide-rich copolymers were isolated as cold-water-soluble products (fraction I). VAA-rich copolymers as well as VAA homopolymer were isolated as fraction III, which was insoluble in hot water and soluble in dimethyl sulfoxide. The intermediate, hot-water-soluble fractions (fraction II) were mixtures of the copolymers and macromonomer. The mole fraction of VAA component in copolymer estimated from the ^{1}H-NMR area ratio was similar to or a little higher than the corresponding one in the monomer feed.

Acrylamide-rich water-soluble copolymers were obtained in rather high yields when the VAA component in feed was less than 0.6 mol % (or 26 wt %). The number-average molecular weights of the copolymers estimated by SEC are listed in the footnote of Table 1. The number of pendant VAA chains in one polymer backbone was calculated from the molecular weights and copolymer compositions to be 6, 8, and 14, respectively, for the copolymers of expt no. 27, 45, and 26. We

Table 2. Copolymerization of VAA Macromonomer (DP_n of Amylose = 40 and 150) with Acrylamide (60 °C)[a]

expt no.	VAA DP_n of amylose	VAA mg	acrylamide, mg	VAA in monomer mol %	VAA in monomer wt %	Me_2SO, mL	H_2O, mL	time, h	yield fr I,[b] %	yield fr II,[c] %	yield fr III,[d] %	VAA in copolymer[e] fr I[b] mol %	VAA in copolymer[e] fr I[b] wt %
47	40	100	400	0.3	20	9	1	34	95	1[f]		0.12	10
48	40	100	233	0.5	30	9	1	34	71	7[f]		0.13	11
43	150	100	400	0.07	20	1.5	1.5	3	46	8	43	0.018	5.9
44	150	100	233	0.12	30	1.5	1.5	3	41	6	47	0.022	7.1

[a] 2,2′-Azobisisobutyronitrile, 0.5 mol %. [b] Soluble in cold water. [c] Soluble in hot water (98 °C). [d] Insoluble in hot water (98 °C). [e] Determined by ^{1}H-NMR. [f] Insoluble in cold water.

Table 3. Amylose−Iodine Complexes[a]

sample	DP_n of amylose	wt % of VAA	UV absorption concn,[b] μ mol/L	λ_{max}, nm	absorbance
macromonomer	24	100	25	521	1.38
	150	100	4.1	603	2.63
poly(VAA-co-AAm)	24	9.7	2.4	450	0.25
	24	32	7.9	497	0.38
	24	32	25[f]	518	0.90
	24	68	12[g]	511	0.43
	24	79	20	506	0.76
	24	89	22	505	0.71
	24	93	23	517	1.12
	40	10	1.5	554	0.23
	40	11	1.7	557	0.30
	150	5.9	0.24	596	0.16
	150	7.1	0.29	591	0.13
hydroxypropylated poly(VAA-*co*-AAm)[c]	24	93	22	498	0.93
hydroxypropylated poly(VAA-*co*-St)[d]	24	83	19	445	0.16
PolyVAA[e]	50		12	613	1.32

[a] Sample (100 mg/L); iodine [52 mg, (205 μ mol/L)] and Me_2SO (20 mL/L). [b] Based on amylose chain. [c] DS = 0.15. [d] DS = 0.30. [e] Prepared from polyVM5A. [f] 310 mg/L. [g] 70 mg/L.

know that SEC is not suitable to estimate the molecular weight of these types of graft copolymers. We can only say that water-insoluble amylose (DP_n = 24) could be dissolved into cold water by incorporating the amylose as the graft component into the polyacrylamide backbone.

Copolymerizations of VAA (DP_n = 40 and 150) with acrylamide are summarized in Table 2. The graft copolymers could be isolated as cold-water-soluble products. The mole percent of VAA in copolymer were lower than that in monomer.

(b) Complex Formation of Poly(VAA-*co*-acrylamide) with Iodine. Table 3 summarizes the wavelength (λ_{max}) and absorbance of the maximum absorption of the amylose−iodine complexes. The λ_{max} and absorbance of amylose−iodine complexes are well-known to depend on the DP_n and concentrations of amylose moieties. The copolymer samples were of different DP_n of amylose moieties and of different weight percent of VAA compositions. Hence, the concentration of iodine and the polymer sample weight were respectively kept constant at 52 mg/L (205 μmol/L) and at 100 mg/L, while the concentrations of amylose chains in the copolymers were changed. Complex formation was attained immediately after mixing, since the UV spectra were almost the same as those taken after 1 day of complexation.

In Figure 2 are plotted the maximum wavelengths (λ_{max}) against the DP_n of amyloses. The curve was drawn using the Banks' data[12] and our newly obtained ones. The plots of VAA macromonomers (DP_n = 24 and 150) were on the curve, suggesting that VAA macromonomer interacted with iodine similarly as amylose. It was reported[13] that 7−8 glucose units at both ends

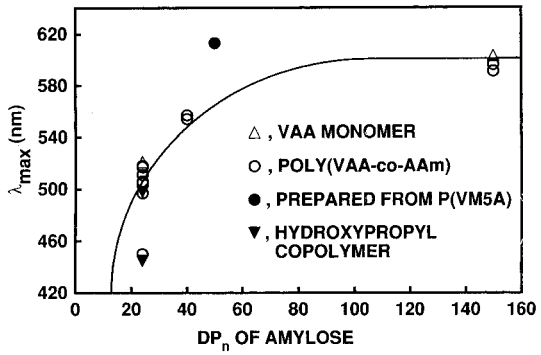


Figure 2. Dependence of the maximum wavelength (λ_{max}) on the DP_n of amylose in various types of amylose-carrying polymer samples.

of amylose chains do not bind iodine because the helical conformation necessary for serving as binding sites may not be attained. The helix formation of amylose chains was little influenced by the presence of the vinylbenzyl group linked to the reducing terminal of amylose chain via an amide group.

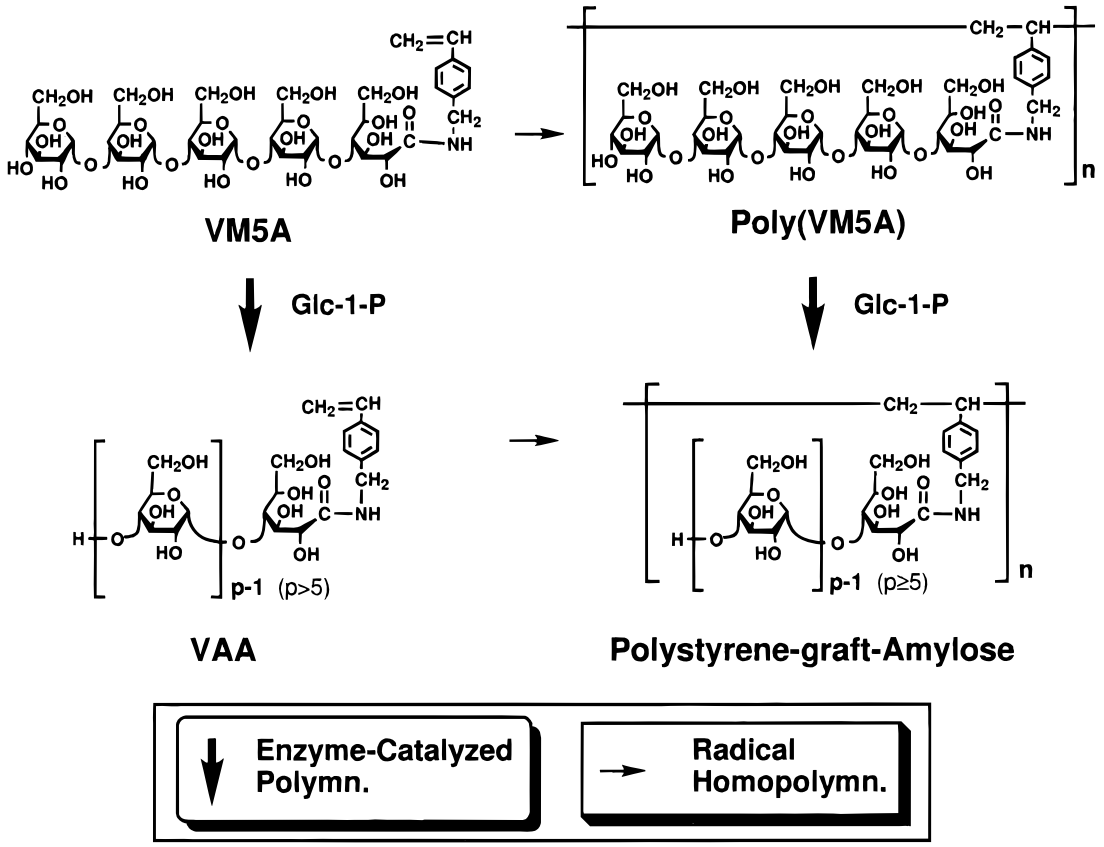
The λ_{max} values of iodine complexes with glycogens and amylopectins were reported to be 460−470 nm and ~550 nm, respectively.[14] The synthetic branched-copolymers (DP_n of amylose chain = 24) possessed intermediate λ_{max} values and hence intermediate amylose chain length between these two naturally occurring branched polysaccharides.

(c) Discussion on the Complexation with Iodine through Side-by-Side Association of Amylose Chains. A number of studies[12−17] have been made on the conformational character of the amylose−iodine complex in aqueous solutions. There seems little doubt that iodine resides within the annular cavity of a helical amylose chain. However, the local conformation is still a matter of dispute. We are interested in the postulation[15,16] that amylose−iodine complexation is promoted through the side-by-side association of helices due to the intra- and intermolecular aggregation of amylose chains.

We have paid attention to some plots of the copolymer samples having DP_n = 24 of amylose. As shown in Figure 3, the maximum wavelengths (λ_{max}) was shifted to higher wavelength with an increase of the concentration of amylose moieties. The absorbance was also increased linearly with the concentration of amylose moieties. These tendencies of the copolymers were similar to those of VAA macromonomers. In contrast, the plots of the maximum wavelengths (λ_{max}) and absorbance against weight percent of VAA compositions were scattered and little tendency was observed.

Complexation was not promoted by an increase of VAA composition but with an increase of amylose concentration. We assumed that, although a high density of amylose moieties was introduced, complexation through side-by-side association was not attained in the present copolymers.

Scheme 2. Two Different Routes to Polystyrene-*graft*-amyloses



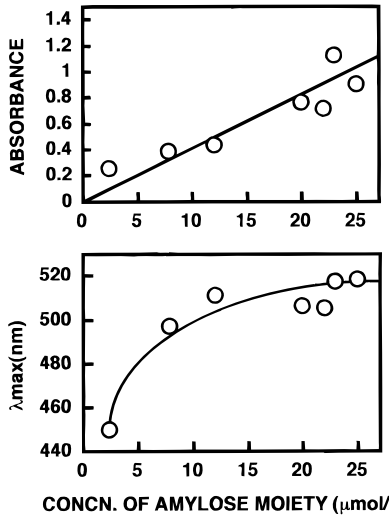


Figure 3. Dependence of the maximum wavelengths (λ_{max}) and absorbances on the concentration of amylose moieties (DP_n of amylose = 24).

3.3. Two Types of Polystyrene-*graft*-amyloses. (a) Preparation via Radical Homopolymerization of VAA and Enzyme-Catalyzed Polymerization onto PolyVM5A. Scheme 2 shows the two different routes to polystyrene-*graft*-amylose. One route is the early enzyme-catalyzed polymerization onto VM5A followed by radical homopolymerization of the resulting VAA macromonomer (DP_n of amylose chain = 24) as mentioned above. Every repeating unit has a hydrophilic polysaccharide graft of relatively uniform length which is connected via an amide group to a hydrophobic styrene moiety. The NMR data of VAA homopolymer (DP_n of amylose = 24) were summarized in the Experimental Section. The main chain methylene and methyne signals were not detected owing to the lack of mobility of the amphiphilic characters, similarly to other oligosaccharide-carrying polystyrene homopolymers reported previously.[9,21,22]

Homopolymerization behaviors of macromonomers have been recently discussed from various aspects by Tsukahara *et al.*[18−20] In spite of unfavorable reactivities of macromonomers, radical homopolymerization of macromonomers gave rather high molecular weight polymers. The production of high molecular weight polymers was interpreted[18] in terms of retardation of bimolecular radical termination between the propagating chain ends which resulted from the high segment density around the propagating radical sites.

Another method is reversed by the order of the polymerization procedures, that is, the radical homopolymerization of VM5A at first and then enzymatic elongation of amylose onto the resulting PolyVM5A. The DP_n of the amylose chain determined by the liberated phosphate was about 50. The NMR spectra were similar to those of the polystyrene-*graft*-amylose described above, except for the absence of the absorption due to the hydroxyl groups of terminal glucose unit at 4.9 ppm (at 27 °C) since the average degree of polymerization of amylose chains was higher.

The molecular sizes estimated by SEC (polystyrene standards) and solution viscosity for the starting PolyVM5A and the resulting polymer are as follows: PolyVM5A, $M_n = 1.24 \times 10^5$ and $M_w = 4.3 \times 10^5$ ($M_w/M_n = 3.4$), $[\eta] = 0.28$ in water and 0.97 in Me_2SO (at 25 °C); the product of enzyme-catalyzed polymerization, $M_n = 0.53 \times 10^5$ and $M_w = 0.86 \times 10^5$ ($M_w/M_n = 1.6$), $[\eta] = 0.79$ in Me_2SO. Both of the apparent molecular size and solution viscosity were decreased in spite of elongation of amylose chains.

(b) Temperature Dependence of ^{1}H-NMR Spectra. Temperature dependence of hydroxyl proton signals in ^{1}H-NMR spectra (100 MH) of maltose, cyclohexaamylose, and amylose were reported by St-Jacques *et al.*[23] in 1976. The anomeric proton signal at 5.1 ppm was kept almost constant, but the hydroxyl proton signals were shifted to higher magnetic fields with an increase of temperature measured. According to their data, upfield migration increased in the order of OH-3 < OH-2 < OH-6, which was interpreted in terms of an intramolecular hydrogen bond from OH-3′ to OH-2.

We re-examined the temperature dependency of amylose (DPn = 34 and 1000) under similar conditions except for the use of a 270 MHz instrument and obtained the different data as follows. Upfield migration increased in the order OH-3 < OH-6 < OH-2. The same tendency was also observed for the present samples of amylose-carrying styrene derivatives (VAA as shown in Figure 1, VM5A, polyVM5A, and polystyrene-*graft*-amyloses via VAA and polyVM5A). The origin of the chemical shift migration with temperature remains a matter of discussion.

(c) Complex Formation. The average DP_n of the amylose chain of an amylose-carrying polymer prepared via polyVM5A was 50. As plotted in Figure 2, its iodine complex showed the λ_{max} at 613 nm. The wavelength was significantly longer than that (~570 nm) predicted for the amylose chain having $DP_n = 50$. We assumed that the effective length of amylose chains was much higher than 50, and hence, the polymer interacted with iodine so strongly. The average chain length of amylose chains effective for iodine complexation was estimated to be 140, by using the equation proposed by Banks:[12]

$$1/\lambda_{max} = 1.558 \times 10^{-3} + 1.025 \times 10^{-2}(1/DP_n)$$

(d) Discussion on Structural Features. Pfannemüller[5] reported that malto-oligosaccharide aldonolactones were attached to a linear backbone chain of polyvinylamine and then phosphorolytic elongation of amylose chains was performed. With relatively low primer concentration along the polymer chain, most of the primer end groups are freely accessible to the potato phosphorylase to grow comb-shaped polymers carrying amylose chains of uniform length. On the other hand, with highly concentrated primers along the polymer chain, all of the primers could not be elongated.

We would like to discuss the distribution of the amylose chain length in the present polymers, although we have no experimental data on the molecular weight distribution. It was reasonble to assume that phosphorylase had to deal with primers so crowded along the present polyVM5A main chain that it was difficult for amylose chains to grow from all the potential sites. If a part of the primers were elongated by the enzyme, the elongated chains became more accessible to the enzyme and unelongated primers became more hindered. If this assumption is true, the elongated amylose chains on polyVM5A possess rather uniform chain length, and sterically hindered primers were left unchanged. In other words, the idealized structure of the copolymer was composed from VAA and VM5A, that is, poly(VAA-*co*-VM5A). One could estimate that about one-third of the primers were elongated to $DP_n = 140$ and about two-third remained unchanged ($DP_n = 5$), which resulted in the average DP_n of 50.

Figure 4 illustrates schematically the synthetic pathway and idealized structures of the two types of polystyrenes having amylose chains as graft components. For convenience, one open circle represents five α-(1-4)-linked glucose residues and one closed circle represents one styrene residue. It is expected to view the figures by imagining that the actual ratio of glucose residues to a styrene residue is much higher than the one illustrated.

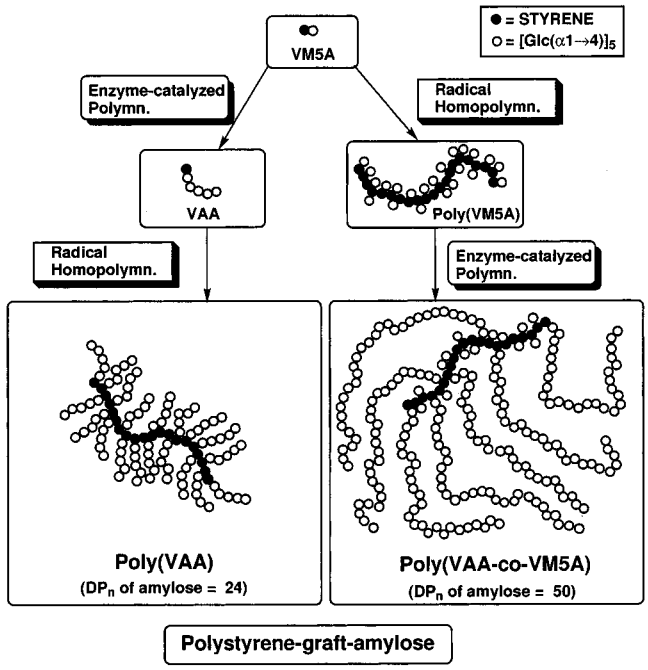


Figure 4. Schematic presentation of the structures of two different types of polystyrene-*graft*-amyloses. The structure of poly(VAA-*co*-VM5A) was drawn by assuming that about one-third of the styrene residues had amylose chains of $DP_n = 140$ and two-third had maltopentaose chains of DP = 5.

3.4. Water-Soluble Polymers Modified with Hydroxypropylation of Amylose. (a) Preparation and Characterization. Water-insoluble copolymer (expt no. 33, 93 wt % of VAA composition in copolymer) was treated with methyloxirane in the presence of sodium hydroxide. The product became soluble in cold water. The methyl signal of the introduced 2-hydroxypropyl ether group was detected at δ 1.06 ppm as a doublet in its ^{1}H-NMR spectrum (D_2O, 70 °C). The degree of substitution (DS) was estimated to be 0.15. In other words, one hydroxypropyl group was introduced into 6.7 glucose residues. Even the rather small substitution could solubilize the hydroxypropylated product in cold water.

The position of hydroxypropyl substitution can be estimated by the ^{1}H-NMR spectrum. The anomeric (H-1) proton absorption was separated into two signals, a larger singlet at δ 5.23 ppm and a smaller singlet at δ 5.48 ppm, the area ratio of which was 0.85:0.15. On the basis of the chemical shift data of methyl ether derivatives of glucopyranoses, the downfield shifted singlet (δ 5.48 ppm) was attributable to the anomeric H-1 signal of a hydroxypropylated glucose unit at position 2 and the large singlet was attributable to the H-1 of the nonsubstituted glucopyranose unit. Since the area ratio (0.85:0.15) corresponded to DS = 0.15, the hydroxypropyl groups were substituted almost exclusively at position 2 of the amylose chain. The hydroxyl group at position 2 possessed the highest reactivity reported for hydroxypropylation of cyclodextrins and cellulose.[24−26]

(b) Complex Formation of Hydroxypropylated Polymer Samples. Figure 2 includes the two data of

2-hydroxypropylated polymer samples. 2-Hydroxypropylated poly(VAA-*co*-acrylamide) (DS = 0.15) induced the λ_{max} at 498 nm and the absorbance = 0.93. The maximum was shifted to lower wavelength from 517 nm and the absorbance was reduced from 1.12 of the original copolymer sample. However, the differences are small, as seen in Figure 2, where the plot of λ_{max} was included in a series of unmodified copolymer samples. The helix-forming ability of amyloses in hydroxypropylated poly(VAA-*co*-acrylamide) was maintained by introducing one hydroxypropyl group per 6.7 glucose residues.

Hydroxypropylated VAA-styrene copolymer with DS = 0.30 induced the λ_{max} at 445 and absorbance at 0.16. The λ_{max} value of this cold-water-soluble polymer clearly deviated from the solid curve (Figure 2). Introduction of one hydroxypropyl group per 3.3 glucose residues disordered the helix structures of amyloses and perturbed the complex formation.

4. Conclusion

(1) Well-defined polysaccharide-carrying macromonomer (VAA: DP_n of amylose = 24 and 150) was synthesized by applying phosphorylase-catalyzed polymerization of glucose 1-phosphate onto maltopentaose-substituted sytrene (VM5A) as a primer.

(2) Homopolymerization of VAA and its copolymerization with acrylamide gave an uncommon type of graft copolymers composed of synthetic polymer as the trunk and polysaccharides as the grafts.

(3) Polystyrene-*graft*-amylose prepared via homopolymerization of VAA (DP_n of amylose = 24) has each repeating unit consisting of a styrene moiety connected with a polysaccharide graft of uniform length.

(4) Polystyrene-*graft*-amyloses obtained via enzyme-catalyzed polymerization onto polyVM5A was interacted with iodine more strongly than expected for the average amylose chain length. We assumed that the amylose chains were elongated from a few VM5A units as primers and sterically hindered VM5A units were left unchanged.

(5) Water-insoluble amylose was solubilized into water by incorporating VAA units into polyacrylamide main chains. Crystallization of amylose was disturbed by the dilution of amylose chains with polyacrylamide backbones.

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