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Synthesis of amphiphilic amylose and starch derivatives

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Abstract

For non-food uses starch generally is modified in order to obtain products with properties suitable for various applications. In the present work, starch and amylose were hydrophobically modified through reactions with long-chain α-alkyl epoxides (C_6 and C_{12}) in DMSO solution, in the presence of NaH as a catalyst. The molar substitution (MS) was calculated from NMR spectra. Derivatives with high as well as low MS values were obtained. In order to reach MS values above 1.5, the reaction had to be run for 150–300 h. Viscosity and GPC measurements indicated that the polysaccharides were degraded in DMSO under the influence of methyl sulfinyl anion, which presumably is the active catalyst.

The derivatives were also characterized by FTIR. The ratio between the peak areas for OH stretching and alkyl stretching vibrations, respectively, in the FTIR spectra, was found to be proportional to MS values determined from NMR spectra.

The solubility of the hydrophobically modified polysaccharide in various solvents was tested. Samples having C_{12}-alkyl side chains and MS > 1 were soluble in toluene. The C_6 derivatives were water soluble up to a MS value of 0.3.

Keywords: Amylose and starch derivatives; alkyl ethers; characterization; molecular weight distribution

1. Introduction

Native starch is one of the most abundant biopolymers on earth and is present in living plants as an energy storage material. Starch is used by man mostly in food applications, but there is a growing interest in the utilization of starch as a renewable raw material for industrial applications (van Bekkum, Röper & Voragen, 1996). Native starches are mixtures of two polyglucans, amylose and amylopectin, consisting of α-D-glucose units linked together in 1,4-position. Amylose is nearly unbranched, having a molecular weight of 10^5–10^6, while amylopectin is highly branched with the branches connected in α-1,6 position of the anhydroglucose unit (AGU). The molecular weight of amylopectin is very high, between 10^6 and 10^7. Amylopectin forms crystalline domains in the starch granules of the living plants, while the linear amylose is believed to be present in the amorphous areas between the crystalline domains. On heating in water, the semicrystalline structure of the granules is broken up, and amylose and amylopectin enter into solution. When a starch solution is cooled down to room temperature, the amylose fraction crystallizes and precipitates out, and can be molecularly dissolved again with difficulty (Aberle, Burchard, Galinsky, Hanselmann, Klingler & Michael, 1997). In aqueous solution, starch molecules have a strong tendency to associate, causing high viscosity.

Another solvent used for dissolution of starch and amylose is dimethyl sulfoxide (DMSO), an aprotic solvent which dissolves starch polymers of any molecular weight. In DMSO the conformation of the amylose molecules is believed to be irregularly helical. (Nakanishi, Norisuye & Teramoto, 1993; Norisuye, 1994, 1995).

For non-food uses starch generally is modified in order to obtain products with properties suitable for various applications, for example, in the pulp and paper industry. The modification can be carried out by chemical methods by introducing small amounts of ionic or hydrophobic groups into the molecules which changes the solution viscosity, association behavior or film forming properties. For uses in the pulp and paper industry derivatives with low degrees of substitution are generally preferred. However, for uses as hydrophobic coatings and adhesives and as blend compatibilizers, highly substituted hydrophobic products may be of value.

The aims of the present work were to synthesize amylose and starch derivatives containing various amounts of hydrophobic groups in order to obtain substances for investigation of structure–property relations and for testing of various applications. Hydroxyalkyl starches can be prepared by

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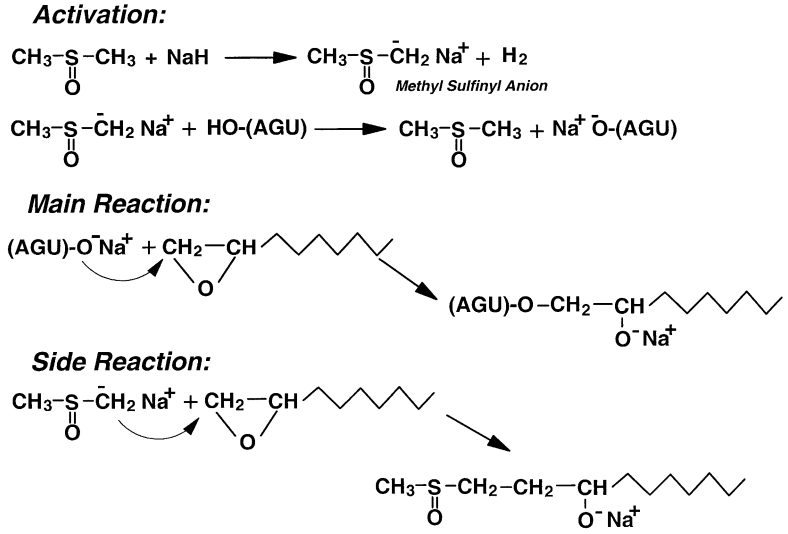

Fig. 1. Reaction of starch/amylose in DMSO with NaH as a catalyst.

reaction of starch with alkylene oxides in the presence of a base. The most common substituents are either hydroxyethyl or hydroxypropyl groups, the reagent being ethylene oxide or propylene oxide, respectively. In the present project, starch and amylose were instead hydrophobically modified through reactions with long-chain alkylene oxides, that is, C6 and C12 α-alkyl epoxides, in DMSO solution, in the presence of a catalyst (Fig. 1). Hydroxyalkyl starches are ethers and these compounds are resistant to cleavage by acids, alkalies and mild oxidizing agents. Salunkhe, Kadam & Jadhav, 1991

2. Experimental

2.1. Materials for synthesis

Amylose from corn (Sigma Chemical Co, USA) was used as received. Native potato starch (250 g) from Lyckeby Starch, Sweden was used as received or after degradation in butanol/HCl. In the latter case, 250 g starch was suspended in 1000 ml 1-butanol (p.a. Merck, Germany)and 200 ml of conc. (36.5% w/w) HCl (Merck, Germany)was added. The mixture was shaken occasionally. After four

Table 1
Reaction of C6 and C12 α-epoxides at ambient temperature with amylose

Experiment	Epoxide	Reaction time (h)	NaH/AGU	Epoxide/AGU	Yield %[a]	MS[b]
Am3-6	C12	6	3/1	1/1	30	0.3
Am3-72	C12	72	3/1	1/1	46	0.5
Am4-24	C12	24	3/1	2/1	41	0.8
Am4-96	C12	96	3/1	2/1	63	1.3
Am5-70	C12	70	3/1	3/1	35	1.0
Am5-168	C12	168	3/1	3/1	59	1.8
Am6-70	C12	70	2/1	2/1	15	0.3
Am6-168	C12	168	2/1	2/1	51	1.0
Am7-168	C6	168	1/1	1/1	20	0.2
Am10-6	C12	6	4/1	3/1	10	0.3
Am10-24	C12	24	4/1	3/1	20	0.6
Am10-48	C12	48	4/1	3/1	27	0.8
Am10-120	C12	120	4/1	3/1	40	1.2
Am11-21	C12	21	3/1	3/1	20	0.6
Am11-46	C12	46	3/1	3/1	33	1
Am11-139	C12	139	3/1	3/1	67	2
Am11-286	C12	286	3/1	3/1	87	2.6
Am12-21	C6	21	3/1	3/1	23	0.7
Am12-46	C6	46	3/1	3/1	27	0.8
Am12-139	C6	139	3/1	3/1	50	1.5
Am12-286	C6	286	3/1	3/1	70	2.1

[a] "Yield" = 100 × (amount epoxide in derivative)/(total amount added).
[b] Estimated from ^{1}H NMR spectra.

Table 2
Reaction of C6 and C12 α-epoxides at ambient temperature with starch and starch modified with HCl in 1-butanol

Experiment	Starch	Epoxide	Reaction time (h)	NaH/AGU	Epoxide/AGU	Yield %[a]	MS[b]
NSBu2-96	Degr starch	C12	96	3/1	3/1	43	1.3
NSBu2-243	Degr starch	C12	243	3/1	3/1	60	1.8
NSBu5-24	Degr starch	C12	24	3/1	3/1	10	0.3
NSBu5-48	Degr starch	C12	48	3/1	3/1	27	0.8
NS-6	Nat. starch	C6	6	3/1	3/1	23	0.7
NS-72	Nat. starch	C6	72	3/1	3/1	53	1.6
NS-120	Nat. starch	C6	120	3/1	3/1	63	1.9
NS-168	Nat. starch	C6	168	3/1	3/1	67	2.0
NS-240	Nat. starch	C6	240	3/1	3/1	77	2.3

[a] "Yield" = 100 × (amount epoxide in derivative)/(total amount added).
[b] Estimated from ^{1}H NMR spectra.

days at room temperature, the degraded starch was filtered from the solution and washed with 70% (v/v) ethanol to remove the acid. The starch was then dried under vacuum at 50°C. (Fox & Robyt, 1992; Ma & Robyt, 1987). Sodium hydride(NaH)was received as a 60% dispersion in mineral oil (Aldrich Chemical Co, UK). The dispersion was washed with *n*-hexane (Merck, Germany) to remove the oil and dried under vacuum before use. Dimethyl sulfoxide dried GR (Merck, Germany)) was used as received.

2.2. Preparation of amylose and starch derivatives

Hexane-washed NaH in amounts given in Tables 1 and 2 was added to 50 ml of DMSO in small doses. The mixture was stirred under N_2 for 24 h under ambient condition. Dried amylose, native starch or butanol/HCl degraded native starch (5 g) was dissolved in 50 ml DMSO at 80°C and then cooled to room temperature and mixed with the NaH/DMSO solution. The solution became very thick and further 20 ml of DMSO was added, and the solution was then stirred for 24 h under N_2 before α-alkyl epoxide was added. The amounts of the different reactants are given in Tables 1 and 2. The alkylation reaction was allowed to proceed under stirring at room temperature. Aliquots were taken out from the reaction mixture at various times up to 243 h after the addition of the alkyl epoxide. The samples were first neutralized with HCl and then the derivatives were precipitated from ethanol, washed with ethanol and dried at 50° C in vacuum. The molecular substitution (MS) for the dried samples was determined by NMR and IR spectroscopy.

2.3. Instruments

FTIR spectra were obtained from films cast onto KBr prisms from toluene or DMSO solutions. After drying at 125°C, the films were analyzed in transmission using a Bruker IFS 66 FTIR spectrometer. ^{1}H NMR spectra were run in DMSO-D_6 using an ARX-500 instrument.

GPC analyses were run at room temperature in THF (Labsscan Ltd, Ireland) concentration 1–2 wt%) on Waters' Styragel columns (10^5, 10^4, 10^3, 500 Å) or 2 Waters' Ultrastyragel linear columns, using differential and viscometry detectors (Dual detector, 250, Viscotek) and a light scattering detector (Chromatix KMX-6) in series. The derivatized samples were dissolved in DMSO and then diluted with THF (ratio DMSO:THF 1/7) before injecting into the GPC instrument.

2.4. Colorimetric determination of amylose in the eluent

The eluate from the GPC columns was collected in 1 ml fractions in small vials and evaporated to dryness under ambient conditions. The residues were dissolved by addition of 0.2 ml DMSO and water was then added to give 4 ml. To 2 ml of this sample, 100 μL of an aqueous phenol solution (80%) was added and then quickly 5 ml of conc. sulfuric acid. The test tube was shaken and after 30 min, the absorbance at 480 nm was measured using a Beckman DB-G spectrometer. A reference sample was prepared by substituting distilled DMSO/water (1/20) for the amylose derivative fractions.

2.5. Viscometry

Intrinsic viscosity at 25°C for amylose and the hydrophobic derivatives in DMSO was obtained from measurements of the specific viscosity of DMSO solutions using capillary viscometery (Ubbelohde viscometer).

2.7. Solubility

The solubility was tested at room temperature using 20 mg derivative/ml solvent. The derivative was regarded as soluble when the sample was completely dissolved after 24 h.

3. Results and discussion

Introduction of hydrophobic groups into hydrophilic starch molecules causes large changes in the physical and chemical properties of the polysaccharide, which becomes amphiphilic in nature. In the present study, starch and amylose were hydrophobically modified by reaction with

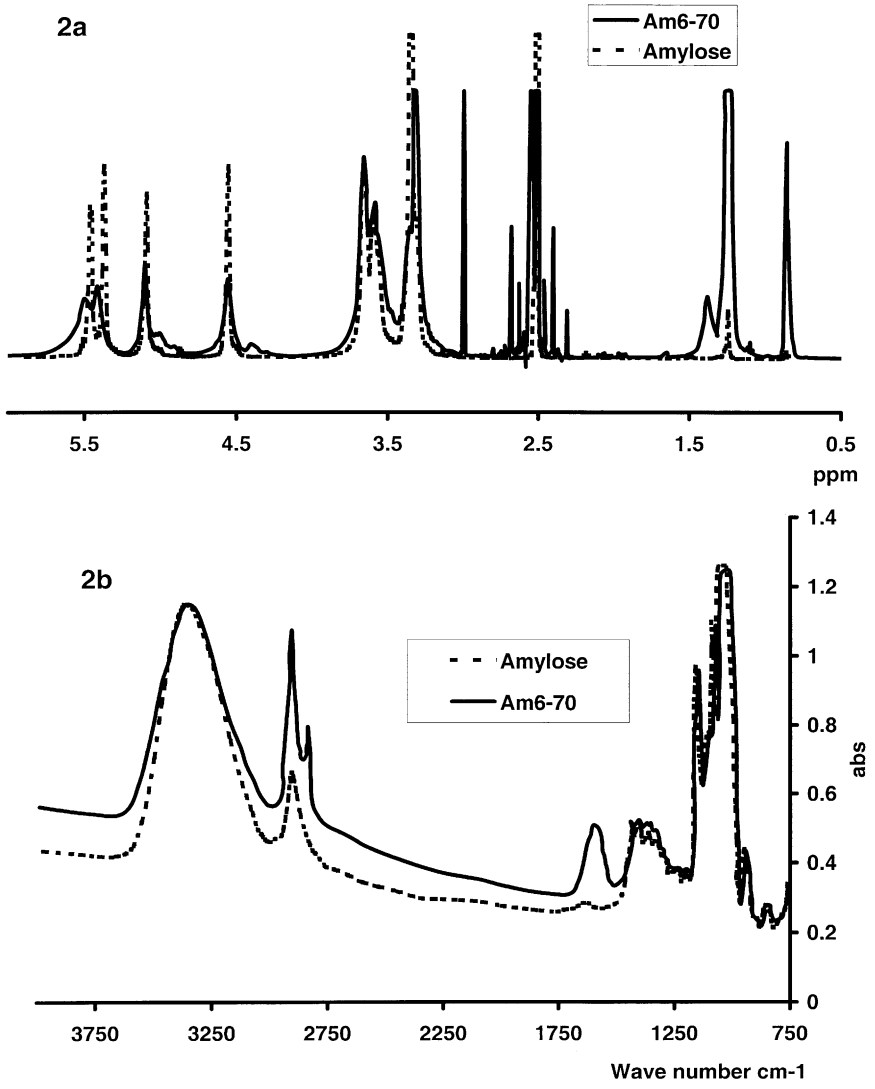

Fig. 2. (a) ^{1}H NMR spectra of amylose and Am6-70 (C12 epoxide). (b) FTIR spectra of amylose and Am6-70 (C12 epoxide).

C_6 and C_{12} α-alkyl epoxides. Epoxides have high reactivity and can be ring-opened under alkaline conditions by nucleophilic reagents, for example, alkoxides. (Morrison & Boyd, 1987) Substitution of starches is generally carried out in aqueous solution or slurry. However, the solubility of both the alkylating reagents and the alkylated products is limited in water, and a two-phase system may develop, especially at high degrees of substitution (DS). It can be anticipated that reaction rates would be rather low in an aqueous system where the low solubility of the alkylating reagent would prevent reaction.

DMSO was selected as the solvent for the alkylation reaction in the present study because amylose and starch (Norisuye, 1995) as well as the alkylated products would be soluble. Of the epoxide reagents the C6 epoxide is fully soluble in DMSO while the C12 epoxide has a limited solubility and, consequently, the reaction system might in that case be heterogeneous. Starch is soluble in DMSO without any appreciable association, and substitution reactions should therefore be random in nature (Steeneken, 1984; Steeneken & Woortman, 1994). In DMSO, the amylose chains are assumed to adopt an irregular helical structure (Nakanishi et al., 1993; Norisuye, 1994) which may decrease the reactivity of hydroxyl groups present in the helical segments. There may also be problems with the compatibility of the reagents with amylose and starch because of the large difference in polarity between the molecules. This might be the case with the longest alkyl chains. Because of the high molecular weight of amylopectin, DMSO solutions are viscous and the transport of the reagents to the reaction sites on the polymer molecules can be a rate-limiting factor rather than the ring-opening reaction.

In base-catalyzed reactions carried out in DMSO, a strong base such as sodium hydride or potassium *tert*-butoxide can be used for activation of the solvent to generate the methyl

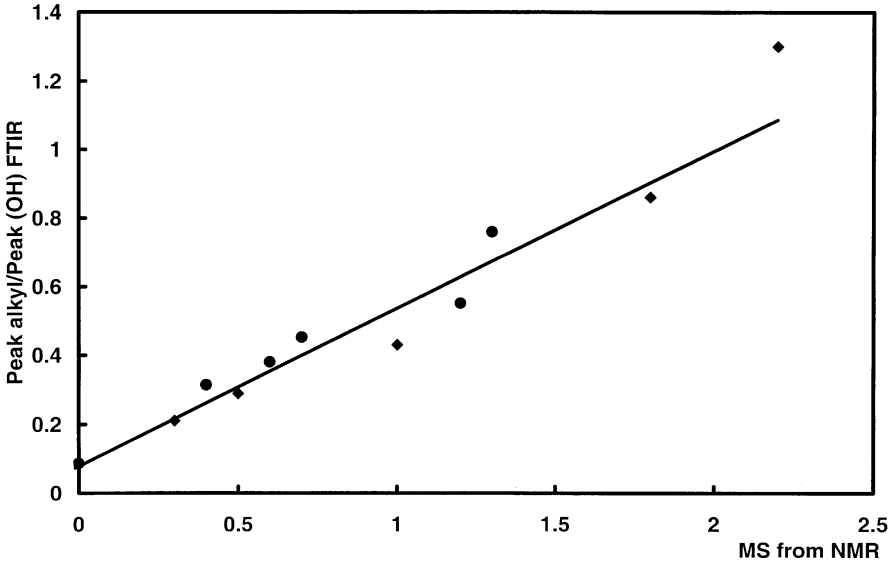

Fig. 3. FTIR peak ratios (3633–3043 cm^{-1})/(3001–2791 cm^{-1}) versus MS determined with NMR spectroscopy for C12 derivatives of amylose (◆) and degraded starch (●).

sulfinyl anion, $CH_3–SO–CH_2^-$, which probably is the active basic species. In the present work NaH was employed, and a reaction scheme is given in Fig. 1 (Ciucanu & Kerek, 1984; Corey & Chaykovsky, 1962). The methyl sulfinyl anion reacts with the OH groups in the polysaccharide to generate alkoxy anions which in turn react with the epoxide ring. In order to reach acceptable overall reaction rates the concentration of alkoxy ions should be high. The methyl sulfinyl anion may also react directly with the epoxide reagent to form a substituted sulfoxide, as also shown in Fig. 1.

The selectivity of the alkylation reaction for the three available positions in each AGU should be a function of the relative acidity/basicity of the secondary and primary hydroxyl groups and the steric shielding effects. Generally, the OH group in the 2-position is considered to be the most reactive one. (Steeneken & Woortman, 1994). As pointed out above, both amylose and amylopectin should be randomly substituted in DMSO. However, a certain blockiness could be anticipated because solubilization of the epoxide by alkyl substituents already present in the polysaccharide molecule would favor reaction of neighboring OH groups.

3.1. Degree of substitution

Starch and amylose were allowed to react with the alkyl epoxides at room temperature after being activated by NaH for 24 h. ^1H NMR spectra were run on the recovered alkylated derivatives and the degree of substitution was calculated from the spectra. An example of an NMR spectra of one derivative is shown in Fig. 2a, together with a spectrum of unsubstituted amylose. The degree of substitution (DS), that is, the number of substituted hydroxyl groups per AGU in the starch polymer, was calculated from the integral of the peak corresponding to CH_2 protons in the epoxide residues, and the integral of the peak corresponding to the single proton in the C1 position of the anhydroglucose unit (Peng & Perlin, 1987). It should be recognized that a secondary hydroxyl group is formed from the epoxide in the alkylation reaction, as evident from Fig. 1, and that this potentially reactive group could be responsible for multiple reaction of epoxide. Thus, it is more correct to refer to substitution values as MS values, i.e. molar substitution, rather than as DS values. Multiple epoxide residues attached to one AGU hydroxyl group cannot be distinguished in the NMR spectra.

It can be noted that at relatively low MS values (<1), there is little change in the relative amounts of the hydroxyl protons at 5.35 ppm (C2–OH), 5.5 ppm (C3–OH), and 4.55 ppm (C6–OH). The reason for this can be that the C2–OH is substituted first, which is to be expected (Steeneken & Smith, 1991), and that the new secondary hydroxyl group derived from the epoxide contributes to the NMR integral in the same region. At MS values >2 the spectra are rather difficult to interpret and as noted above it cannot be determined if oligomerization of the epoxide occurs.

FTIR spectra of amylose and one of the C12 amylose derivatives are shown in Fig. 2b. The samples were dried before running the spectra. It was found that the ratios between the integral of the peak at 3633–3043 cm^{-1} (OH-stretching) and that at 3001–2791 cm^{-1} (alkyl stretching) was proportional to the MS values determined from the NMR spectra, as shown by the graph in Fig. 3. The graph was used in a fast method to monitor the development of the MS of the derivatives during the reactions.

One of the objectives of the study was to prepare a range of hydrophobic starch and amylose derivatives having high as well as low MS values. High MS values were obtained

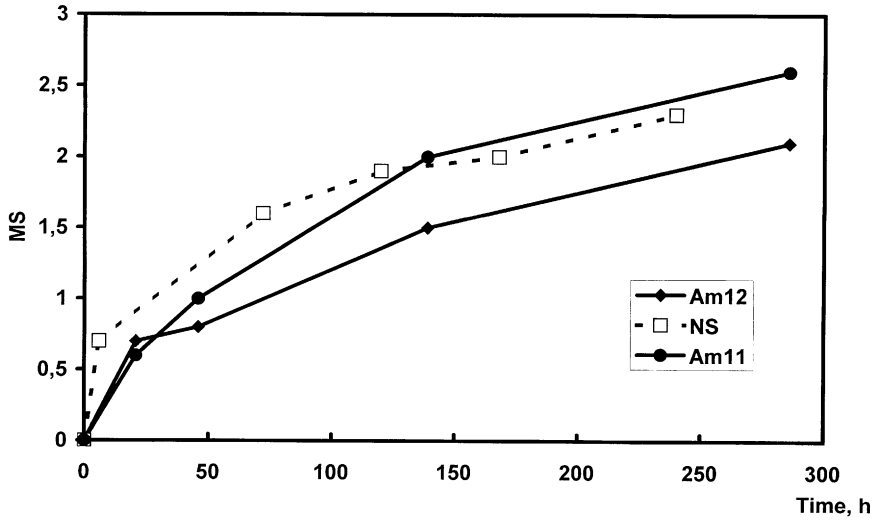

Fig. 4. MS as a function of time for reaction of native starch and amylose at ambient temperature with C6 or C12 epoxides, respectively, in DMSO with NaH as a catalyst. Amylose, C6 epoxide (Am12, ◆); starch, C6 epoxide (NS, □); and amylose, C12 epoxide (Am11, ●).

only at fairly long reaction times, even though the amounts of catalyst and reagent were high (3–4 mol/AGU). As seen in Tables 1 and 2, and Fig. 4, MS values above 1.5 could be reached at reaction times of about 50 h for both the C6 and C12 epoxides, even though the C12 epoxide has a limited solubility in the reaction mixture. The latter observation can be explained by a neighboring group effect from the alkyl chains already present in the polysaccharide, which would help to solubilize the C12 epoxide. After 50 h, the reaction rates decreased substantially, as seen in Fig. 4, and the reaction had to be run for 150–300 h to reach MS values between 2 and 3. The low reaction rates observed at long reaction times presumably are a consequence of the most reactive hydroxyl groups being substituted first, and of the epoxide reagent being consumed.

A likely side-reaction should be consumption of the epoxide through anionic polymerization initiated by the methylsulfinyl anion, as indicated above (Fig. 1). However, no epoxide oligomers were observed under the conditions used for the substitution reaction.

3.2. Solubility and molecular size

The solubility of the hydrophobically modified polysaccharide samples in different solvents was tested, and the results are reported in Table 3. Samples having C12 alkyl side chain and MS >1 were soluble in toluene. When the C6-epoxide was used for derivatization, the products were water soluble up to an MS value of 0.3.

The viscosity of the starting materials, i.e. amylose and starch, and that of the derivatives, were measured in DMSO solution. As shown in Fig. 5, the intrinsic viscosity for amylose decreased with time on treatment with NaH at room temperature without any epoxide being present. This observation indicates that amylose is degraded in DMSO under the influence of the methyl sulfinyl anion, with a decrease in the molecular weight of the polysaccharide. However, it is also quite obvious from Fig. 5 that reaction with the epoxide reagent decreased the intrinsic viscosity of the material to a higher extent as compared to the situation for pure amylose degradation. At higher MS values,

Table 3
Solubility of amylose and starch derivatives in various solvents

Experiment	Polysaccharide	Epoxide	MS	Water	Ethanol	DMSO	Toluene
Am4-24	Amylose	C12	0.8	−	−	+	−
Am4-96	Amylose	C12	1.3	−	−	+	+
Am5-168	Amylose	C12	1.8	−	−	+	+
Am7-168	Amylose	C6	0.3	+	−	+	−
Am11-139	Amylose	C12	2	−	−	+	+
Am12-139	Amylose	C6	1.5	−	+	+	−
NSBu2-96	Deg starch	C12	1.3	−	−	+	+
NSBu3-96	Deg starch	C12	0.6	−	−	+	−
NS-6	Nat. starch	C6	0.7	−	−	+	−
NS-72	Nat. starch	C6	1.6	−	Partly	+	−
NS-168	Nat. starch	C6	2.0	−	Partly	+	−

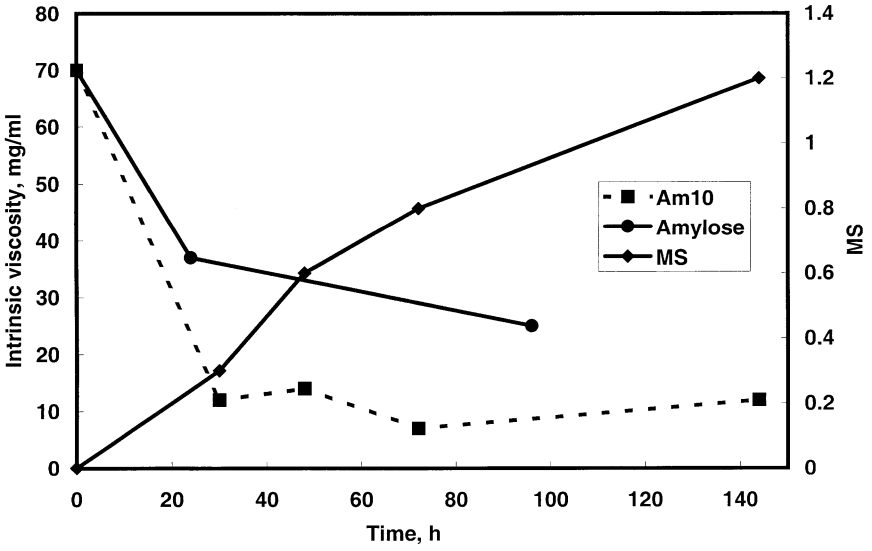

Fig. 5. Intrinsic viscosities versus time for reaction of amylose in DMSO with NaH (Amylose, ●), and with NaH and C12 epoxide (Am10, ■), at 25°C. For the latter reaction the corresponding MS values are given (MS, ◆). Amylose was treated with NaH in DMSO (3 mol NaH/mol AGU) without epoxide present.

intramolecular interactions within the hydrophobic derivatives would give rise to a more compact molecular conformation, in which the alkyl side chains would be shielded by the polysaccharide backbone from interaction with DMSO. The resulting small hydrodynamic volume could then explain the low intrinsic viscosity values of the derivatives.

Gel permeation chromatography (GPC) is generally used to determine molecular size. However, there are problems to find a good common solvent for, on the one hand, amylose and starch, and on the other, for the hydrophobic derivatives (Chen, Fringant & Rinaudo, 1997). It was observed that if the high-MS derivatives were first dissolved in DMSO and then diluted with THF to a DMSO/THF ratio of 1:7, GPC analyses could be performed with THF as eluent. As shown in Fig. 6, there is an increase in the elution volume with increasing reaction time and MS. Viscometry and LALLS detectors indicated that no high molecular weight aggregates were present, as shown in Fig. 7. It seems likely that degradation of the starch or amylose molecules takes place at the reaction conditions used, but intramolecular association of the derivatives in the polar medium with reduction of the hydrodynamic volumes has also to be considered.

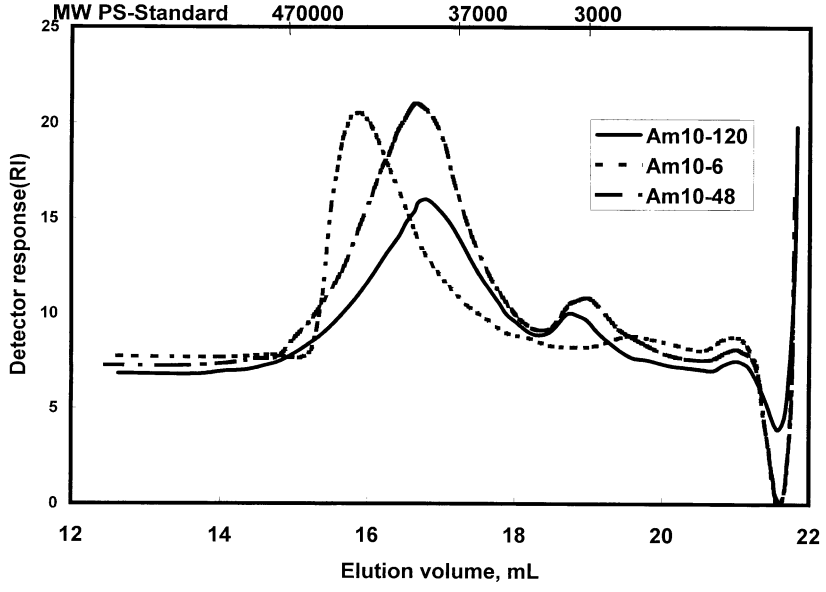

Fig. 6. GPC traces run in experiment Am10 (amylose, C12 epoxide) at different reaction times. Two waters ultrastyragel linear columns. RI detector. Eluent: THF, flow rate: 1 ml/min. The samples were dissolved in DMSO/THF 1:7 before injection.

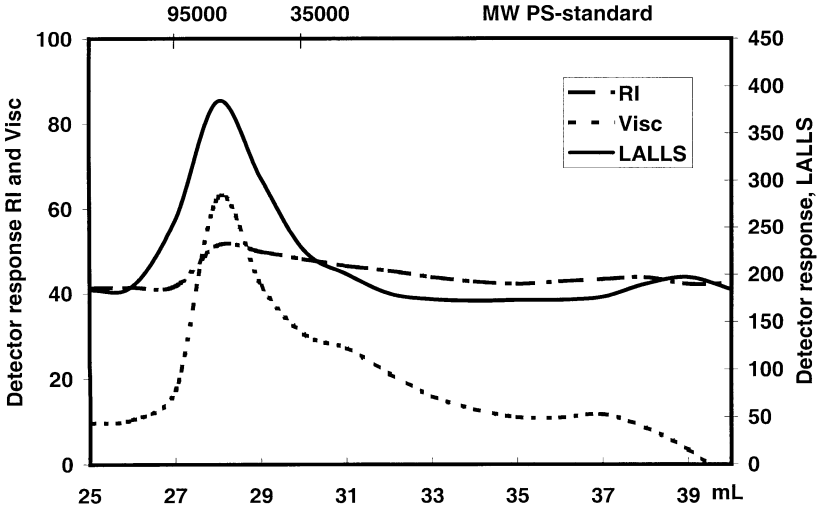

Fig. 7. GPC traces for sample Am10–48 (amylose, C6 epoxide) collected with different detectors (RI, viscosity and LALLS). Waters' μStyragel columns. 10^5, 10^4, 10^3 and 500 Å. Eluent: THF, flow rate: 1 ml/min. The samples were dissolved in DMSO/THF 1:7 before injection.

The polysaccharide concentration in a solution can be determined by a method proposed by Dubois, Gilles, Hamilton, Rebers & Smith., 1956. For a number of derivatives the effluent from the GPC column was collected in fractions, and the polysaccharide content was determined and compared to the RI detector signal from the GPC run, which should be proportional to the total concentration of polymer. As can be seen in Fig. 8, the RI signal parallels the carbohydrate content, which means that there are no major differences in MS values between high and low molecular weight fractions.

4. Conclusions

Starch and amylose can be hydrophobically modified by reaction with aliphatic α-epoxides in DMSO solution, with various degrees of substitution (MS) attainable. C12 α-epoxides give products soluble in toluene when MS ≥ 1, in contrast to the C6 α-epoxides. The C6 derivatives are soluble in water at MS ≤0.3. In the presence of NaH, amylose and amylopectin molecules degrade in DMSO at room temperature with a reduction of the molecular weight.

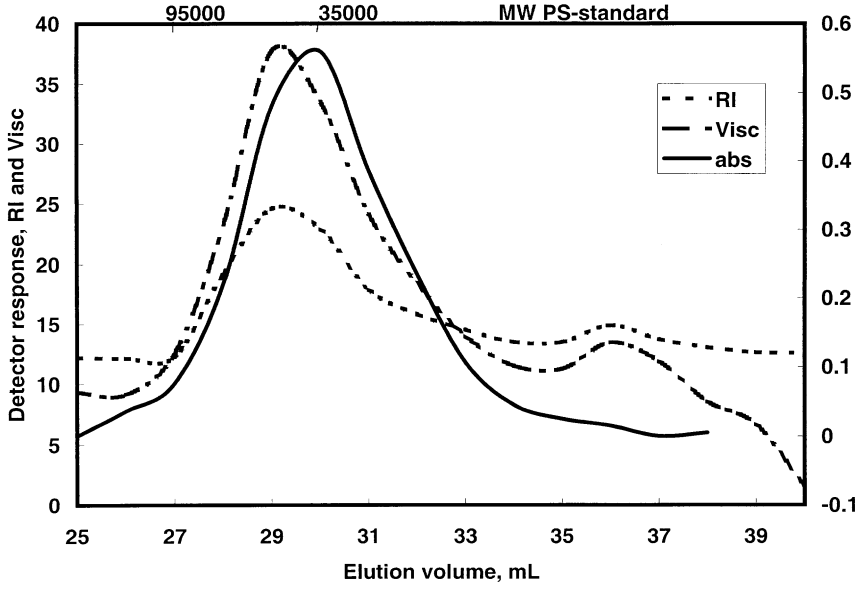

Fig. 8. GPC traces for sample Am10–48 (amylose, C12 epoxide) collected with different detectors (RI, viscometry). Carbohydrate content was determined on the eluate by phenol/sulfuric acid method and reported as absorbance at 480 nm (abs). Waters' μStyragel columns. 10^5, 10^4, 10^3 and 500 Å. Eluent: THF, flow rate: 1 ml/min. The samples were dissolved in DMSO/THF 1:7 before injection.

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