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Biodegradable amylose-g-PLA glycopolymers from renewable resources

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ABSTRACT

The synthesis of biodegradable PLA-grafted amylose copolymers has been achieved in three steps to control their architecture as best as possible. First, amylose was partially protected with various silylating agents, especially N,O-bis-(trimethylsilyl)acetamide (BSA). The influence of the silylating agent chemistry and of the BSA/OH molar ratio on the protection yield have been investigated. Furthermore, the reactivity order of the different OH functions towards silylation has been evaluated. The second step is based on the "grafting form" strategy: polylactide (PLA) grafts were generated by the Ring Opening Polymerization (ROP) of D,L-lactide from the free remaining OH groups carried by the partially silylated amylose. Finally, the silylether groups cleavage was tested under various conditions to obtain the PLA-grafted amylose (A-g-PLA), while avoiding backbone and grafts degradation. In addition, two polyelectrolytes, i.e., LiCl and $NaNO_3$ have been used to facilitate the SEC analysis of native amylose.

1. Introduction

Since many years, biodegradable polymers have become of interest as substitution materials to polluting petrochemical ones. These compounds have been classified into two groups that are agro-polymers and biopolyesters (Averous, 2004). The latter are biodegradable polyesters such as PLA (polylactic acid), PCL (poly(ε-caprolactone)) or PHA (polylactide, polyhydroxyalkanoate) for instance. In case of PLA, the monomer is derived from renewable resources and lactic acid is produced by the hydrolysis of PLA. Agro-polymers (polysaccharides, proteins, etc.) can be obtained in a common way from renewable resources at low production costs and are degradable by environment. However, renewable starch for instance has severe limitations due to its higher water sensitivity and relatively poor mechanical properties when compared to petrochemical polymers. In order to improve its properties and overcome these difficulties, some authors tried either to plasticize starch (plasticized starch is also called "thermoplastic starch") or to blend it with a petrochemical polymer (e.g., PVA (Sreedhar, Chattopadhyay, Sri Hari Karunakar, & Sastry, 2006) or PE (Dole, Averous, Joly, Della Valle, & Bliard, 2005; Jang, Huh, Jang, & Bae, 2001; Matzinos, Bikiaris, Kokkou, & Panayiotou, 2001; Wang, Yu, & Yu, 2004)) or a biopolyester e.g., PLA or PCL (Belard, Dole, & Averous, 2005; Dean, Yu, Bateman, & Wu, 2007; Ke, Sun, & Seib, 2003; Matzinos, Tserki, Kontoyiannis, & Panayiotou, 2002; Preechawong, Peesan, Supaphol, & Rujiravanit, 2005). Unfortunately, such a blend usually results in a non-miscible one. Various strategies targeting an increase of the adhesion between the two phases have thus been explored in order to improve the performances of such blends. Basically, all these strategies consist in the synthesis of one compatibilizer obtained *in-* or *ex-situ*.

In case of starch/PLA blends, these compatibilization strategies have been brought together in a previous paper (Schwach et al., in press). Interestingly, few attempts to generate PLA grafts from native starch (unplasticized) (Gong, Wang, & Tu, 2006) have been published but only one paper deals with a PLA growth from amylose (Ohya, Maruhashi, & Ouchi, 1998). Actually, starch is mainly composed of amylopectin and amylose. Amylose is almost a linear polymer composed of α-D-glucose units linked with (1 → 4) bonds, while amylopectin is a highly branched polymer composed of α-D-glucose units linked with (1 → 4) and (1 → 6) bonds. Though Ohya et al. (1998) outlined an interesting study, they used low molecular weight amylose (GPC standard grade, $\overline{M_n} = 1.5 \times 10^4$ g/mol) or degraded native amylose from potatoes with $\overline{M_n}$ around 7×10^3 g/mol. Moreover, they did not study in detail the synthetic scheme they used, even though each step is a key to control the architecture of the obtained copolymer.

Herein, we report on the synthesis of biodegradable amylose-g-PLA copolymers (A-g-PLA), which could be used as *ex-situ* produced compatibilizer agent in starch/PLA blends. More precisely, the objective of this work was to attain a structural control of these copolymers. These glycopolymers (here copolymer based on a polysaccharide part) were derived from native amylose and were synthesized through a three-steps procedure (Scheme 1): (i) partial silylation of the hydroxyl groups carried by amylose chains; (ii) ring-opening polymerization (ROP) of D,L-lactide initiated from all the remaining hydroxyl groups on the partially silylated

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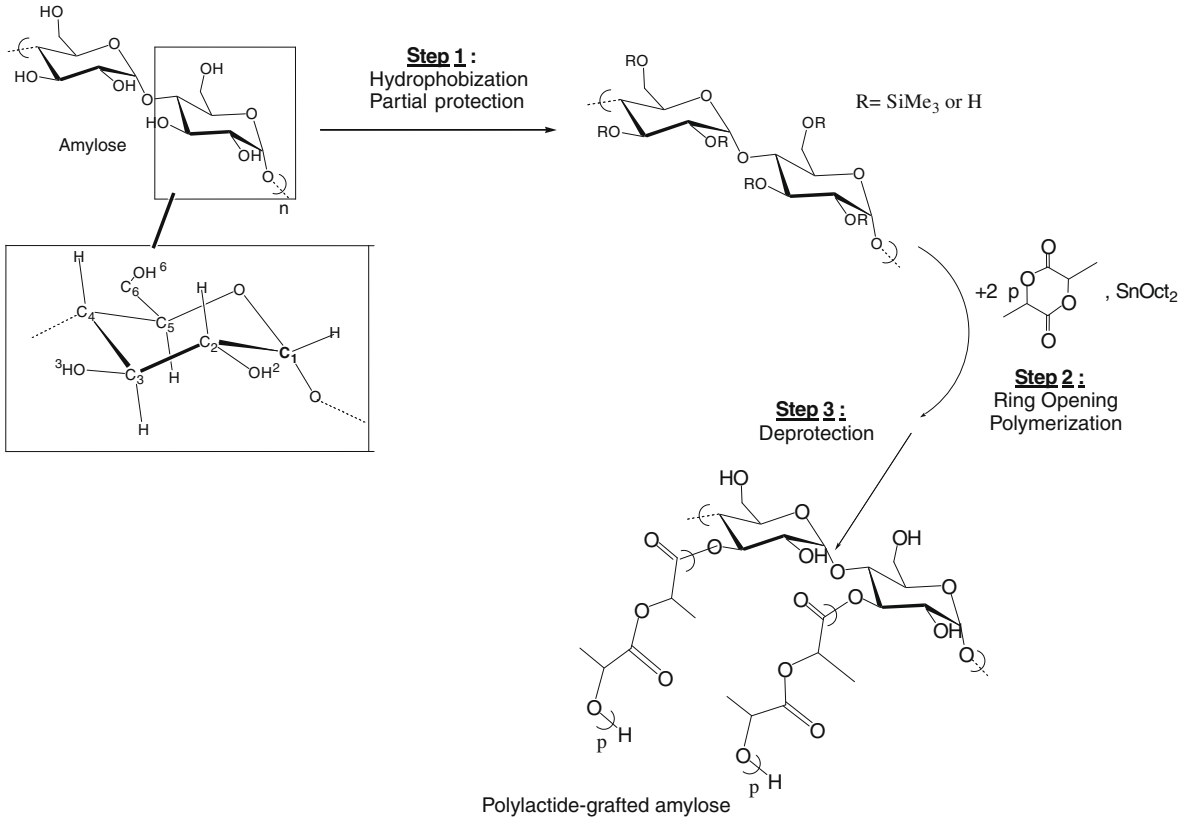

Scheme 1. Synthetic scheme of A-g-PLA. (The insert shows one glucosidic unit).

polysaccharide;and (iii) silylether deprotection under mild conditions. Various experimental conditions have been tested to silylate the amylose in order to check the reactivity order of each alcohol function carried by the glucosidic units but also to obtain a large range of silylation yields.

2. Experimental part

2.1. Materials

Native amylose from potatoes was purchased from Fluka, dissolved in dimethyl sulfoxide (DMSO), then precipitated from THF and dried under a reduced pressure at 100 °C for one night. No significant degradation of the polysaccharide chains was evidenced under these drying conditions as attested by SEC–MALLS. 1,1,1,3,3,3-Hexamethyldisilazane (HMDS) and N,O-bis-(trimethylsilyl)acetamide (BSA) were purchased from Sigma and Aldrich, respectively. Chlorotrimethylsilane (TMSCl) and 1-trimethylsilyl imidazole (TMSI) were obtained from Fluka; stannous octoate ($SnOct_2$) was purchased from Aldrich. All these reagents (Fig. 1) were used without any purification. After dilution with dry toluene, $SnOct_2$ solution was stored in glass ampoule under nitrogen. D,L-Lactide from Lancaster was recrystallized twice with dry toluene and dried under vacuum just before use. Toluene and DMSO were refluxed over CaH_2. After distillation, solvents were stored under nitrogen atmosphere. Just before use, these solvents were respectively dried over polystyryllithium and CaH_2, respectively, and distilled again. Dialysis membranes were purchased from Spectra Por (MWCO: 6000–8000).

2.2. Silylation

Forty milliliters of DMSO were added onto 1 g of amylose under a nitrogen flow with a previously dried canula. Silylation was carried out in a previously dried and nitrogen purged round-bottomed two-necked flask equipped with a stopcock. The amylose was left to solubilize for 4 h at 50 °C (or 1 h at 100 °C then 1 h at 50 °C). Then, the desired amount of silylating agent was added using a syringe and the reaction was kept at 50 °C during 20 h. At the end of the reaction a mixture made up of a yellow gel composed by the predominant part of the silylated amylose and of a liquid containing a little amount of modified polysaccharide has been obtained. The polysaccharide isolated from the crude liquid phase exhibits similar protection yield than that from the gel. After cooling and

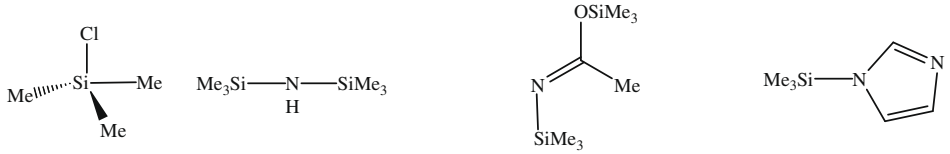

Fig. 1. Silylating agents used in this study.

separation, the yellow gel was solubilized in THF (10 mL of THF/g of native amylose) then precipitated twice from cold ethanol and dried under vacuum. In some cases, evaporation of DMSO was performed before precipitation, but this was subsequently rejected because of amylose degradation (see later).

2.3. Silylation yield

Silylation yields (t), which give the number of trimethylsilyl-ether groups per 100 OH functions, were estimated from ^{1}H NMR spectrum of protected amylose in $CDCl_3$ (Fig. 2A) or in DMSO-d_6 (in case of low silylation yield, not shown in this paper) using Eq. (1) where A_{OSiMe3} and $A_{anomeric\ H}$ are the respective areas of the trimethylsilyl groups (at 0.13 ppm) and of the anomeric protons centered at 5.12 ppm.

$$\text{Silylation yield}(t,\ \%) = \frac{A_{OSiMe_3}}{A_{anomeric\ H}} \times \frac{100}{27} \quad (1)$$

From this data, one can readily determine the degree of substitution (DS), i.e., the number of protected hydroxyl functions per glucosidic unit. DS was calculated from the silylation yield using Eq. (2):

$$DS = \frac{3 \times \text{silylation yield}\,(\%)}{100} \quad (2)$$

The protection yield values were confirmed after reaction between the free remaining OH functions of partially silylated amylose with an excess of trichloroacetylisocyanate (De Vos & Goethals, 1986). Such a reagent is known to react almost instantaneously with water and primary or secondary alcohols. If some water reacts with this isocyanate, trichloroacetamide is produced, which is characterized by two singlets at 5.7 and 6.6 ppm in ^{1}H NMR ($CDCl_3$) (Fig. 2B). The reaction of amylose OH functions with this isocyanate leads to one multiplet [4.7–5.5 ppm]. This multiplet corresponds not only to the anomeric protons but also to the glucosidic ones carried by carbons linked to a urethane function (proved by 2D NMR, not shown here). The other multiplet [3–4.5 ppm] corresponds to the glucosidic protons carried by carbons linked to a $-OSiMe_3$ group (Fig. 2B). Consequently, the global area (called A) from 3 to 5.5 ppm corresponds systematically to seven protons (six glucosidic protons and the anomeric one). With this indirect method, silylation yields could be estimated from the ^{1}H NMR spectrum shown in Fig. 2B using either Eq. (3) or (4). A and A_{NH} are the respective areas of the multiplets between 3 and 5.5 ppm and of the urethane protons [8.5–9.5 ppm]

$$\text{Silylation yield}(t,\ \%) = 100 - \frac{A_{NH}}{A} \times \frac{7 \times 100}{3} \quad (3)$$

$$\text{Silylation yield}(t,\ \%) = \frac{A_{OSiMe_3}}{A} \times \frac{7 \times 100}{27} \quad (4)$$

Similar silylation yields were obtained regardless of Eqs. (1), (3), or (4).

2.4. Polymerization

The procedure has been adapted from one of our previous paper dealing with the synthesis of dextran-g-PLA (Nouvel, Dubois, Dellacherie, & Six, 2004). A dried partially silylated amylose was dissolved in dry toluene. The solution was heated at 100 °C, then transferred to a D,L-lactide toluene solution heated at 100 °C. A defined volume of $SnOct_2$ was added to the resulting solution and the polymerization temperature was kept constant for 20 h. The reaction was stopped by addition of a catalytic amount of acidic methanol. The D,L-lactide conversion was determined by ^{1}H NMR analysis ($CDCl_3$) of the crude product. The pure product was recovered by several precipitations/filtrations and dried under vacuum. Polylactide-grafted (silylated amylose) was analyzed afterward by ^{1}H NMR in $CDCl_3$ (Fig. 3A). The silylation yield estimated after the polymerization was similar to those previously observed after the first step as already reported (Nouvel et al., 2004). Considering that all the free hydroxyl groups carried by the partially silylated amylose exhibited the same reactivity and initiated the polymerization of the D,L-lactide (because of the rapid equilibrium between free OH and tin alkoxides *in situ* produced by reaction between OH and $SnOct_2$), the $\overline{DP_n}$ of PLA grafts ($\overline{DP_{n\ graft}}$), expressed in lactyle units (molecular weight 72 g/mol), could be estimated by Eq. (5), or (6):

$$\overline{DP}_{n\ graft} = \frac{A_{CH_3\ PLA}}{A_{glucosidic\ H}} \times \frac{2}{3 \times 1 - t/100} \quad (5)$$

$$\overline{DP}_{n\ graft} = \frac{A_{CH_3\ PLA}}{A_{OSiMe_3}} \times \frac{3 \times (t/100)}{1 - t/100} \quad (6)$$

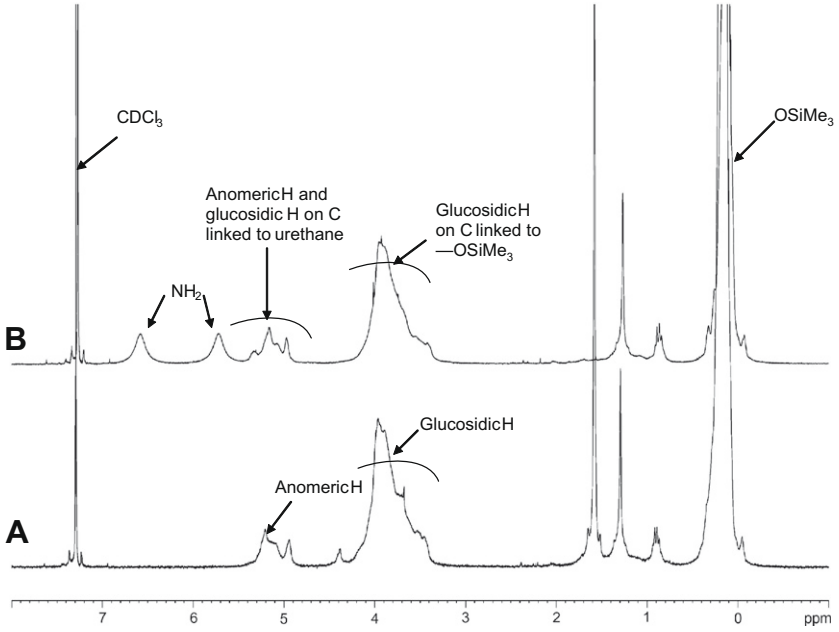

Fig. 2. ^{1}H NMR spectra ($CDCl_3$) of partially silylated amylose before (A) and after (B) reaction with trichloroacetylisocyanate.

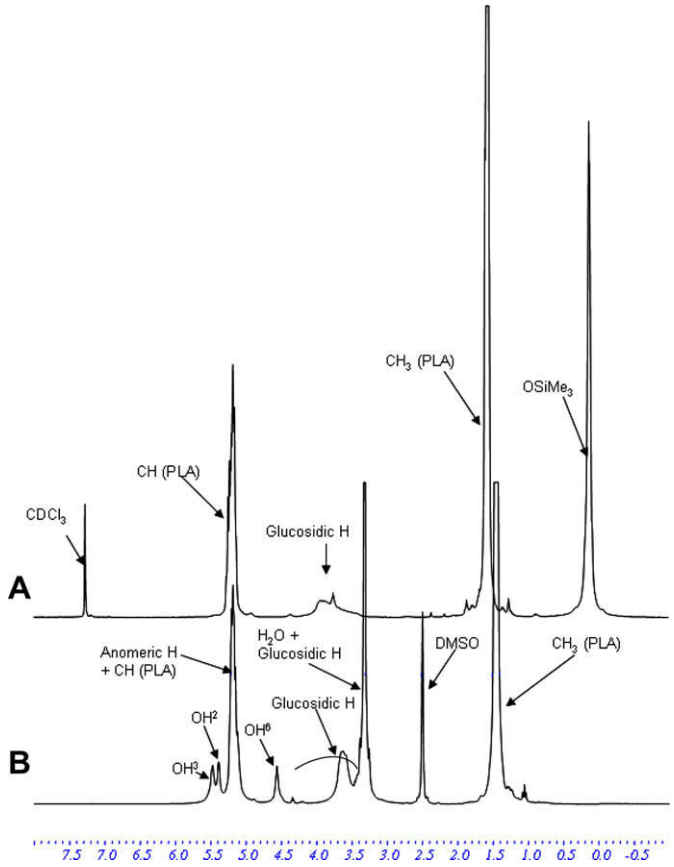

Fig. 3. ^{1}H NMR spectra of polylactide-grafted (silylated amylose) (A, $CDCl_3$) and of amylose-g-PLA (B, DMSO-d_6, run 3, Table 2).

where $A_{\text{glucosidic H}}$, A_{OSiMe_3} and $A_{CH_3\ PLA}$ are the areas of glucosidic protons (from 3.3 to 4.3 ppm), of trimethylsilyl groups (at 0.13 ppm) and of methyle protons of polylactide (around 1.6 ppm), respectively (Fig. 3A). The area of PLA methyle protons has been preferred for calculation instead of that of methyne ones (around 5.2 ppm), those latter showing a similar chemical shift to anomeric protons.

The average number of PLA grafts (y) per 100 glucosidic units could be estimated by Eq. (7).

$$y = 3(1 - t/100) \times 100 \tag{7}$$

After checking that each silylated copolymers were characterized by only one population in size exclusion chromatogram, their PLA weight fraction (F^{PLA}) was estimated from the ^{1}H NMR spectrum ($CDCl_3$, Fig. 3A), using Eq. (8).

$$F^{PLA} = \frac{\text{PLA weight}}{\text{PLA weight} + \text{Amylose weight}} = \frac{72 \times A_{CH_3\ PLA}}{72 \times A_{CH_3\ PLA} + 162 \times \frac{A_{\text{glucosidic H}}}{2}} \tag{8}$$

where 72 and 162 are the molecular weights of the lactyle and glucosic units, respectively.

2.5. Deprotection

Silylated amyloses and silylated glycopolymers have been dissolved in THF (1 g/20 ml THF). A catalytic amount of HCl aqueous solution with respect to the number of "–$OSiMe_3$" groups was then added. After 24 h at room temperature, the products were recovered by precipitation with ethanol, filtration and drying under vacuum. In like manner, the F^{PLA} could be estimated from the ^{1}H NMR spectrum in DMSO-d_6 (Fig. 3B) applying Eq. (8) and was in good agreement with that of silylated copolymers as also proved for Dex-g-PLA glycopolymers (Nouvel et al., 2004).

2.6. Characterization techniques

^{1}H NMR spectra were recorded using a Bruker Avance 300 apparatus in DMSO-d_6 or $CDCl_3$. FT-IR spectra were recorded using a Bruker Tensor 27 spectrometer. Size exclusion chromatography (SEC) was performed at room temperature using a Merck L-6200 HPLC pump equipped with a DG-1310 degasser, a guard column and a PLgel column (5 μm, 300 × 7.5 mm). Elution (0.7 mL/min) was dually monitored by MALLS (λ = 690 nm) and differential refractometry (Waters 410). Amylose solutions (10 mg/mL) were analyzed with (DMSO + $NaNO_3$ (0.1 M) or DMSO + LiCl (0.5 M)) as eluent, while THF was used for silylated products and amylose-g-PLA exhibiting a high PLA weight fraction. Refractive index increments of 0.034 and of 0.087 were used for amylose in (DMSO + $NaNO_3$ (0.1 M)) and for silylated ones in THF, respectively. A refractive index of 0.054 was used for PLA in THF. The values of dn/dc of each grafted copolymers were measured using the online refractometer and accurate concentrations.

3. Results and discussion

The direct synthesis of amylose-g-polylactide copolymers (A-g-PLA), that means using the amylose hydroxyl groups as initiator groups for the ROP of lactide (without the first protection step) did not give satisfactory results because of the poor solubility of this polysaccharide in organic solvents (Ohya et al., 1998). Moreover, to prevent the ROP of lactide occurring under heterogeneous conditions, one can hydrophobize amylose chains by reversible protection. Consequently, the ROP has been carried out under homogeneous conditions in an appropriate solvent. Thus, we choose to synthesize the A-g-PLA glycopolymers using a three-step strategy depicted in Scheme 1. Based on the "grafting from" concept, this synthetic pathway involves (i) a partial silylation of the polysaccharide hydroxyl functions, (ii) the ROP of D,L-lactide promoted by all the remaining OH groups of the partially protected amylose and finally (iii) a deprotection step. Such a strategy enables copolymer architecture in terms of grafts and backbone lengths and grafts number to be controlled provided each step is perfectly controlled.

3.1. Protection step

3.1.1. Silylation

Among the methods to protect hydroxyl groups, reversible silylation has been selected in this study. The advantages of such a procedure were reported in a previous paper (Nouvel et al., 2002). Among organic solvents of native amylose, DMSO seemed to be the best solvent to allow the silylation reaction. Trimethylsilylation of amylose has been already tested for a long time (Harmon, De, & Gupta, 1973; Keilich, Tihlarik, & Husemann, 1968; Ohya et al., 1998). However, to the best of our knowledge, no in-depth study of the silylation of amylose has been reported so far. In this study, our aim was to get a controlled silylation, leading to a partially silylated amylose exhibiting a controlled number of residual OH functions. For that reason we studied the influence of various parameters, such as the nature of the silylating agent, the molar ratio between the silylating agent and the amylose OH functions (noted silylating agent/OH molar ratio in this paper). The addition of a co-solvent, which is a solvent of the partially silylated amylose, has also been studied. The silylation was carried out at 50 °C during 20 h. Finally, all the silylated products were characterized by SEC, IR and ^{1}H-NMR spectroscopy.

On one hand, four silylating agents (TMSCl, HMDS, TMSI and BSA) (Fig. 1) were investigated either alone or in pairs to introduce the trimethylsilylether functions as protector groups. As reported in Table 1, when using one silylating agent alone, BSA is the most effective silylating agent to reach a silylation ratio higher than 90%. TMSCl and HMDS, which are common silylation agents did not give such a high protection yield. The same conclusion was drawn in case of dextran (Nouvel, Dubois, Dellacherie, & Six, 2003). Although TMSI has been described as a very effective silylating agent (Bu & Rhee, 2000; Harabagiu et al., 2004), it leads to a weak silylation yield in case of amylose.

As TMSCl has been reported to be used as an electrophilic catalyst for silylation with HMDS, we investigated the influence of such a catalyst on the silylation of amylose carried out with BSA or HMDS. As shown in Table 1, TMSCl activated the silylation by HMDS to reach an 86% silylation yield. Explanation of this result was given by Nagy, Borbely-Kuszmann, Becker-Palossy, and Zimonyi-Hegedus (1973). Interestingly, this silylating mixture led to a full protection on dextran (Nouvel et al., 2002) under the same experimental conditions. Surprisingly, no increase of the silylation yield was observed when TMSCl has been mixed to BSA.

On the other hand, we studied the influence of the BSA amount in comparison with the alcohol functions carried by the amylose chain. As shown in Fig. 4, increasing the BSA/OH molar ratio enhances the silylation yield. For example, using a BSA/OH molar ratio of 0.5 led to a DS of 2.5. From this degree of substitution, the solubility of partially silylated amylose in DMSO vanishes. The product becomes soluble in most organic solvents, particularly in toluene, this latter being used in the second step. Thus, during the reaction, the medium remained homogeneous when a BSA/OH ratio smaller than 0.25 was applied, whereas for ratios above 0.5, a gelification of the medium was rapidly noticed. No kinetics study was made in order to optimize the reaction, but it is probable that the silylation yield reached after a few hours was appreciably equal to the final one. Furthermore, it was observed that 2 mol of BSA per mole of OH functions were necessary to reach a silylation yield higher than 90%.

To avoid gel formation while protecting amylose with high DS, a co-solvent, that means a solvent of the partially silylated amylose, was added in the medium at the beginning. Two solvents were investigated (THF and toluene) while the silylation was carried out using BSA. As reported in the case of dextran (Nouvel et al., 2003), the use of a co-solvent allows a small increase of the silylation yield (Table 1).

Fig. 5 shows the IR spectra of partially silylated amyloses synthesized at various BSA/OH ratios. The absorption band at around 3300 cm^{-1} was assigned to the O–H stretching vibration of hydroxyl groups of amylose. The absorption bands at 840 and 1265 cm^{-1} were assigned to the Si–O–C and Si–CH_3 vibrations, respectively. With the increase of BSA/OH ratio, we noticed a reduction of the absorbance at around 3300 cm^{-1}, while bands at 840 and 1265 cm^{-1} enhanced. These results confirm that the silylation of amylose succeeded and that the degree of substitution of amylose increased with BSA/OH ratio. Molar masses of partially silylated amyloses have been evaluated by SEC, using THF as an eluent (not shown here). Contrary to what one might think, the molecular weights of the partially silylated amyloses did not increase logically with the DS. The explanation for this will be given in Section 3.2.

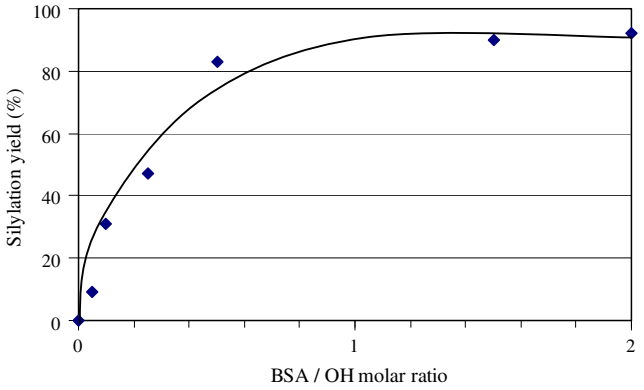

Fig. 4. Effect on BSA/OH molar ratio on the silylation yield.

3.1.2. Alcohol reactivity order toward silylation

To evaluate the reactivity of each alcohol function carried by the amylose chain, a detailed attribution of the ^1H NMR spectrum in DMSO-d_6 has been achieved (Cheetham & Tao, 1998; Ortiz, Reguera, & Fernandez-Bertran, 2002; Sugiyama et al., 2000). We assumed that each glucosidic unit exhibits the same accessibility and that the reactivity of each OH function does not change while other OH groups were modified. The study of ^1H NMR spectra of various silylated amyloses when different DS allows us to determine the reactivity order of the three OH functions toward silylation (Fig. 6). The doublet corresponding to the OH carried by the C_2 (see insert in Scheme 1), which is noted in the following text as OH^2, was vanishing more rapidly than the others OH when the protection yield increased (proved after peaks deconvolution). This highlighted the higher reactivity of OH^2 and the weakest reactivity of OH^3. The reactivity order of each amylose OH function toward silylation was thus obtained as $OH^2 > OH^6 > OH^3$, which is in agreement with previous results (Lee, Kim, & Jun, 1999; Luby & Kuniak,

Table 1
Effect of the silylating reagent.

Silylating reagent[a]	Silylation yield (%)[b]	DS[c]	$\overline{M_n}$[d] (g/mol)	$\overline{M_w}$[d] (g/mol)	I
HMDS (2)*	81	2.4	255,000	320,000	1.25
BSA (2)	92	2.8	370,000	462,000	1.25
TMSI (2)*	67	2.0	220,000	300,000	1.36
TMSCl (2)	75	2.2	155,000	210,000	1.35
HMDS (1.8) + TMSCl (0.2)	86	2.6	180,000	260,000	1.44
BSA (1.8) + TMSCl (0.2)	85	2.6	315,000	410,000	1.30
BSA (2)[e]	94	2.8	320,000	450 000	1.40
BSA (2)[f]	95	2.9	340,000	440,000	1.30

[a] The silylating reagent/OH molar ratio is given in brackets.
[b] Calculated from Eq. (1).
[c] Calculated from Eq. (2).
[d] Values for the deprotected silylated amyloses (see text). SEC was carried out with DMSO + 0.1 M $NaNO_3$ as eluent. The values of the purified amylose average molecular weights in the same eluent were $\overline{M_n}$ = 460,000 g/mol and $\overline{M_w}$ = 640,000 g/mol.
[e] Initial medium = DMSO/THF (9/1 v/v).
[f] Initial medium = DMSO/Toluene (9/1 v/v).
* DMSO was removed from the crude medium by evaporation under reduced pressure, before purification of the product by precipitation.

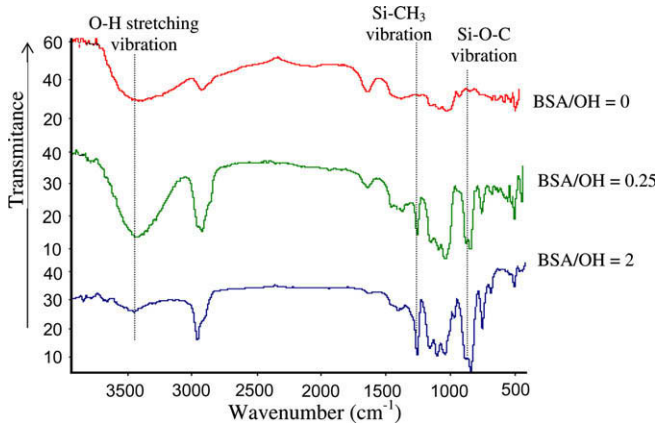

Fig. 5. IR spectra of silylated amyloses prepared with various BSA/OH molar ratios.

1979). In conclusion, at DS close to 3, the remaining hydroxyl functions would be mainly OH^3. In other words, when almost quantitatively silylated amylose was used as macroinitiator for the ROP of lactide (next step), the produced PLA grafts were probably linked mainly via the C_3 amylose.

3.2. Amylose degradation during the silylation

3.2.1. SEC of native amylose

Usually native amylose is eluted in pure DMSO and the SEC–MALLS chromatograms exhibit aggregates, leading to high molar masses (>10^6 g/mol). According to some papers (Chuang & Sydor, 1987; Radosta, Haberer, & Vorwerg, 2001; Zhong, Yokoyama, Wang, & Shoemaker, 2006), low molecular weight electrolytes like LiCl and $NaNO_3$ have been tested to screen interactions, which are responsible for such aggregation. Consequently, these two salts were added to DMSO at different concentrations. Each mixture was used both to dissolve native amylose and as a SEC eluent. As expected, these salts improved the chromatogram by preventing aggregation, yielding to sensible molar masses. No variation of elution volumes was observed when the salt concentration was higher than 0.1 M $NaNO_3$ or 0.5 M LiCl. But surprisingly, elution volumes varied with the nature of the salt (Fig. 7). Without any doubt, this indicates that these polyelectrolytes do not screen the interactions between amylose chains in the same way.

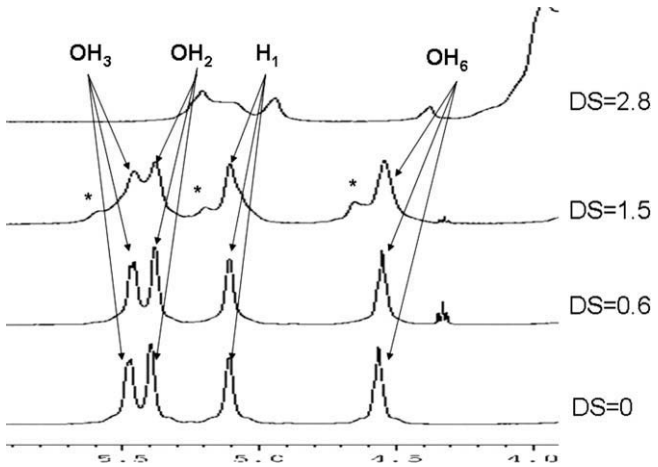

Fig. 6. ^{1}H NMR spectra (from 4 to 6 ppm) of various partially silylated amyloses. Degrees of substitution (DS) are given on the left for each spectrum. DMSO-d_6 was used as a solvent for DS < 1.5, while $CDCl_3$ was used for higher DS. ∗ = Glucosidic H on C linked to –$OSiMe_3$ group (proved by 2D NMR).

Similarly, the precipitation of native amylose by THF, leading to purified amylose, probably fractionates the polysaccharide or removes aggregates and consequently improves again the chromatogram. After such a precipitation, similar SEC chromatograms have been observed when LiCl or $NaNO_3$ are added to DMSO eluent (Fig. 7). In the following, DMSO + 0.1 M $NaNO_3$ has been chosen as eluent. The values of the average molecular weights of purified amylose in this eluent were $\overline{M_n}$ = 460,000 g/mol and $\overline{M_w}$ = 640,000 g/mol.

3.2.2. Deprotection of silylated amylose

As mentioned above, a difference between the molar masses of purified and silylated amyloses was observed in SEC–MALLS. This difference is certainly due to the degradation of the polysaccharide chains during the silylation step as reported for dextran (Nouvel et al., 2003). To check this hypothesis, partially silylated amyloses were deprotected under very mild conditions, using a catalytic amount of HCl with respect to the number of silylether groups.

Previously, the mildness of such conditions have been confirmed to avoid the degradation of amylose chain (thus to not degrade the final glycopolymer during the ultimate step of the Scheme 1). DMSO and THF were investigated because they are solvents of native and highly silylated amyloses, respectively. On the first hand, when purified amylose was dissolved in DMSO, degradation of the polysaccharide chains was observed by SEC–MALLS whatever the acidic conditions we tested (Table 2). On the other hand, when the purified amylose was suspended in THF, the decrease of amylose chain length was observed only for very acidic conditions. No degradation appears with milder conditions, i.e., with r (molar ratio between HCl and –$OSiMe_3$ groups onto polysaccharide chains) equal or less than 0.1, even during 24 h reaction. These milder conditions were applied for the deprotection of 95% silylated amylose. The quantitative deprotection was observed for r = 0.1 after 24 h. Contrary to previous reports (Ohya et al., 1998), the deprotection could not be achieved using neutral THF/Methanol mixture during 48 h. The stability of PLA chains under these mild acidic conditions have been already published (Nouvel et al., 2004).

3.2.3. Amylose stability during the first step

Using the mild deprotection conditions (HCl 0.1 M, r = 0.1), the various partially silylated amyloses were deprotected and analyzed by SEC–MALLS. By comparison with purified amylose, a predominant degradation of the polysaccharide was observed when BSA or TMSCl have been used (Table 1). Significant degradation was also observed when DMSO was evaporated before the precipitation of crude silylated amylose. By the way of example, although HMDS was a less damaging silylating agent than BSA (Nouvel et al., 2003), heating the crude silylation medium to evaporate DMSO promotes the degradation of the polysaccharide (Table 1). Moreover, the presence of a co-solvent or increasing the BSA/OH molar ratio, which proved to be necessary to obtain higher silylation yields, increases the degradation of amylose chain as well. Anyway, we have to point out that a reduced molecular weight polysaccharidic backbone would have a favorable impact on the efficiency of A-g-PLA as a compatibilizer agent for starch/PLA blends, by making the diffusion of the glycopolymer to the interface easier.

3.3. Ring opening polymerization (ROP) of D,L-lactide from the silylated amylose

The second step of the Scheme 1 consists to use the partially silylated amylose as a multifunctional macroinitiator for the ROP of D,L-lactide. 0.03 equivalents of a tin activator ($SnOct_2$) compared to the free remaining alcohol functions were used according to some previous obtained results (Nouvel et al., 2004). Highly silylated

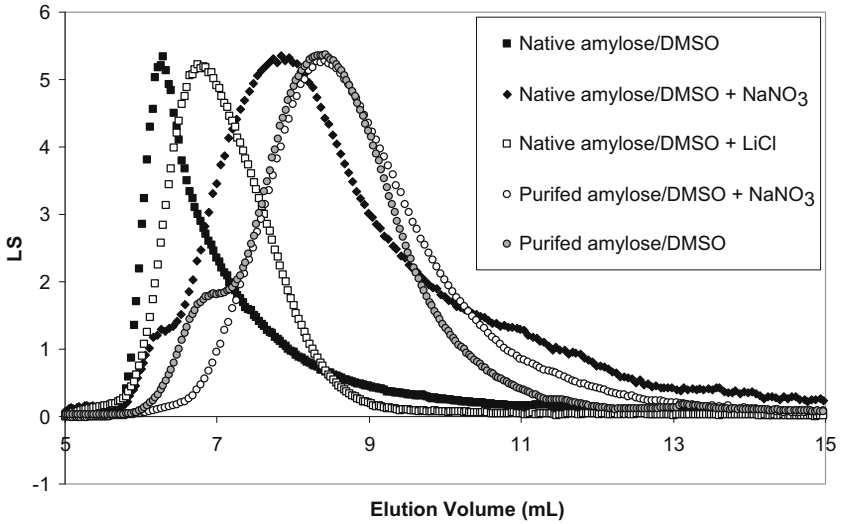

Fig. 7. Evolution of SEC chromatograms (MALLS detector) of native and purified amyloses depending on the eluent. The SEC curve of purified amylose obtained with DMSO + LiCl as eluent perfectly overlaps that observed with DMSO + $NaNO_3$ (not shown for clarity).

amyloses being soluble in organic solvents like toluene, we used this solvent to carry out the ROP in homogeneous conditions. $SnOct_2$ is well known to reduce the extent of the transesterifications (Penczek, Duda, & Libiszowski, 1998), which are secondary reactions occurring during this ROP and consequently hampering the control of the macromolecular parameters of the expected copolymer. Among the numerous initiators of the ROP of lactide, $SnOct_2$ has been found to be one of the most suitable for control of polymerization (Baran, Duda, Kowalski, Szymanski, & Penczek, 1997) as its propagation rate is high while the level of transesterification reactions remains limited. Temperature is also essential to control the polymerization and must be bellow 120 °C (Kowalski, Duda, & Penczek, 2000; Kricheldorf, Kreiser-Saunders, & Boettcher, 1995; Ryner, Stridsberg, Albertsson, von Schenck, & Svensson, 2001). Consequently, the ROP reported in this paper was carried out at 100 °C in toluene. Moreover, it is generally accepted that a fast alcohol–alkoxide interchange compared to the propagation rate exists. Thus, all the free remaining hydroxyl groups of the macroinitiator were activated and initiated the polymerization, even if a low amount of $SnOct_2$ was used as an activator.

Different polylactide-grafted silylated amyloses were thus produced and characterized by ^{1}H NMR in $CDCl_3$ and by SEC–MALLS using THF as an eluent (Table 3). After purification, a decrease in elution volume was systematically observed after the PLA grafts growth. All the copolymer chromatograms reported here showed only one population, which proved that no uncoupled PLA chains were mixed with the silylated copolymer (Fig. 8). In fact, if uncoupled PLA chains existed, their elution volume would be higher than that of the copolymer (checked with various PLA standards) and was not observed after purification of the crude product. Consequently, the ^{1}H NMR spectrum (Fig. 3A in $CDCl_3$) allowed us to estimate the $\overline{DP}_n$ of each PLA graft using Eq. (6), and thus the PLA weight fraction (F^{PLA}) using Eq. (8). The ^{1}H NMR spectrum exhibited both peaks characteristic from the PLA part (methyle protons at 1.6 ppm, methyne protons around 5.1 ppm), from the trimethylsilyl groups (0.1 ppm) and from the amylose chain. Results are presented in Table 3. The copolymers differ one from the others by the average number of PLA grafts per 100 glucosidic units (y), the average length of grafts and by the F^{PLA}. As shown in Table 3, the ROP was stopped deliberately before complete conversion of lactide to limit the transesterifications probability. Actually, this probability increases when monomer concentration is close to the equilibrium one (Duda & Penczek, 1990). All the results obtained by ^{1}H NMR as well as by SEC–MALLS demonstrated both the efficiency of the polylactide grafting onto the silylated amylose

Table 3
Polylactide-grafted silylated amylose parameters. t (%) is the silylation yield of the amylose (see Table 1 for experimental conditions). y is the number of PLA grafts per 100 glucose units. $\overline{DP}_{ngraft}$ is the average degree of polymerization of each PLA graft, expressed in lactyle unit (molecular weight 72 g/mol). Consequently, $\overline{M}_{ngraft} = 72\ \overline{DP}_{ngraft}$. F^{PLA} is the PLA weight fraction in the silylated copolymers.

Runs	t (%)	y	$\overline{DP}_{ngraft}$	$\overline{M}_{ngraft}$	F^{PLA}	Conversion yield (%)
1	83	51	74	5400	0.94	53
2	92	24	188	13,500	0.95	61
3	98	6	20	1440	0.35	–
4	67	99	196	14,000	0.99	70
5	95	14	100	7200	0.86	–
6	85	45	134	9600	0.96	44

"–" means not evaluated.

Table 2
Stability of purified amylose and deprotection of highly silylated amylose (t = 95%) under various acidic media. r is the molar ratio between HCl and –$OSiMe_3$ groups onto the polysaccharide chain. In the case of purified amylose, r was considered as the molar ratio between HCl and all the OH functions carried by the amylose chain.

	Purified amylose in			Silylated amylose (t = 95%) in THF	
	THF		DMSO		
Acidic conditions	2 h[a]	24 h[a]	24 h[a]	2 h[a]	24 h[a]
HCl 1 M; r = 0.1	Degradation	–	–	–	–
HCl 1 M; r = 0.02	Degradation	–	–	–	–
HCl 0.1 M; r = 0.1	No degradation	No degradation	Degradation	Partial cleavage of $OSiMe_3$	Quantitative cleavage of $OSiMe_3$
HCl 0.1 M; r = 0.02	No degradation	No degradation	Degradation	–	Partial cleavage of $OSiMe_3$

[a] Reaction time.

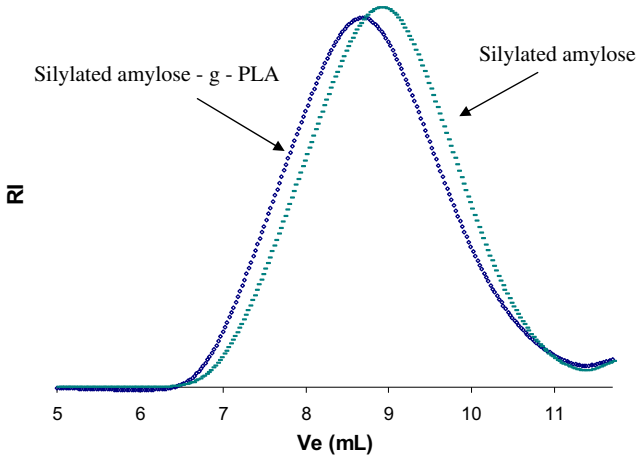

Fig. 8. SEC chromatograms of 92% silylated amylose (Table 1) and the derivated silylated amylose-g-PLA (run 2, Table 3).

backbone and the absence of significant transesterifications (absence of homopolylactide chains).

3.4. Deprotection of silylated copolymers

The hydrolysis of the silylether groups carried by the amylose backbone has been realized after checking that both the amylose and the PLA chains were not degraded under the applied conditions. As proved above, no degradation of amylose chains was observed under mild conditions, i.e., using HCl 0.1 M with *r* (molar ratio between HCl and $-OSiMe_3$ groups onto polysaccharide chains) equal or less than 0.1, even during 24 h reaction. Moreover with HCl 0.1 M, r = 0.1 was necessary to quantitatively cleave the $-OSiMe_3$ groups carried by the silylated amylose. Consequently, these milder conditions have been applied for the deprotection of PLA-grafted silylated amylose in THF. Crude products were precipitated by ethanol and dialyzed against ethanol for 48 h.

The various deprotected copolymers (A-g-PLA) were characterized by ^{1}H NMR (Fig. 3B) using DMSO-d_6. The absence of peak at approximately 0.13 ppm ($-OSiMe_3$ groups) clearly demonstrated the quantitatively deprotection of these brush-like copolymers. The chemical shifts of methyle and methyne protons of PLA were observed at around 5.25 and 1.45 ppm, respectively, when the spectrum exhibits characteristic peaks of amylose protons. From this spectrum, the weight fraction of PLA (F^{PLA}) in the recovered amylose-g-PLA was estimated using Eq. (8). As DMSO-d_6 solubilizes both amylose and PLA parts, F^{PLA} estimated in this deuteried solvent was similar to that of PLA-grafted silylated amylose, as we observed in case of dextran-g-PLA (Nouvel et al., 2004). These results prove that no degradation of both PLA grafts and amylose backbone has been evidenced.

4. Conclusion

During this study, various amylose-g-PLA copolymers were synthesized using a three-step synthetic pathway: (i) a partial silylation of hydroxyl functions carried by the amylose chain, (ii) the polymerization of D,L-lactide from all the remaining hydroxyl functions on the modified polysaccharide, and finally (iii) the deprotection of the silylether groups. Each step was carefully studied, specially the protection one. Depending on the nature and amount of silylating agent used, both the protection yield and the degradation of the amylose chain varied. Various acid conditions have been tested for the deprotection step, but only mild conditions have been selected to cleave the silylether groups, while preventing the degradation of both amylose and PLA parts. Consequently, the control of each step allowed us to tune the macromolecular parameters of the copolymers. Thus, various amylose-g-PLA containing from 35 to 99 wt% of PLA have been produced. The use of such amylose-g-PLA glycopolymers to improve the adhesion between PLA and starch will be reported in the future.

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