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Synthesis of silica-bound amylose by phosphorolytic elongation of immobilised maltoheptaosyl hydrazides

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Abstract—Maltoheptaoside-alkoxysilane anchor molecules were synthesised by fusing aliphatic ω-$Si(OEt_3)$ hydrazide linkers with maltoheptaose. After immobilisation of the primers on porous silica, support-bound amylose was synthesised by phosphorolytic synthesis. The hydrazone linkage as a pre-formed cleavage site allowed removal and subsequent characterisation of immobilised amylose, which showed a broad molecular weight distribution. Under HPLC conditions, amylose assumed a non-helical conformation, making surface interactions and not complexation the primary separation mechanism. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Amylose is a linear polysaccharide composed of α-1–4 linked glucose moieties, which, together with the highly branched amylopectin forms the polysaccharide component of starch. Native starch,[1–3] derivatives of amylose and cellulose,[4–6] as well as cyclodextrins[7] have been used for chromatographic enantioseparation. Amylose can adopt a helical conformation which is able to form inclusion complexes with various guest compounds.[8–12] Similar complexation mechanisms are known for cyclodextrins,[13] which have long been used for chiral recognition and encapsulation of compounds.[14–16] Carbamate derivatives of polysaccharides, adsorbed on or covalently linked to solid supports are frequently used for chiral separation.[17,18] Silica-bound amylose carbamate was prepared by enzymatic synthesis followed by immobilisation and derivatisation.[19] However, inclusion complexes of amylose have not yet been exploited for chromatography. Here, synthesis and immobilisation of oligosaccharide primers on porous silica,[20] followed by solid-phase enzymatic chain elongation, is reported. The synthetic route ensured that silica-bound amylose was attached to the support via its reducing end, allowing for maximal conformational flexibility of the immobilised polysaccharide chain. Immobilised amylose could be removed via breaking of a pre-formed cleavage site, allowing analysis of the synthetic process. Properties of the new phases in high-pressure liquid chromatography (HPLC) were tested.

2. Results and discussion

Bifunctional maltooligosaccharide-silane linkers were synthesised from ω-unsaturated carboxylic acids by conversion to the corresponding hydrazides (Fig. 1).[21]

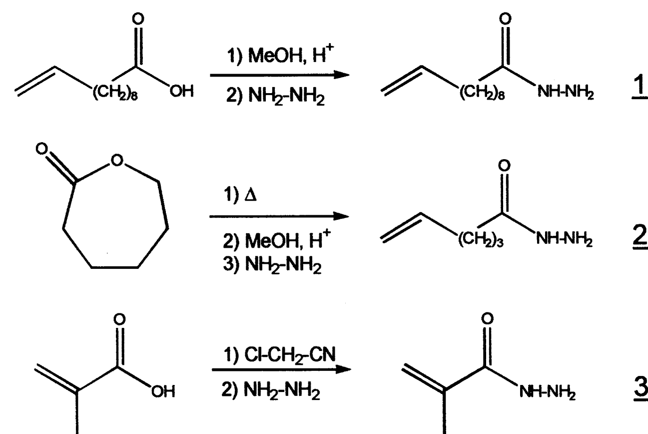

Figure 1. Synthesis of ω-unsaturated hydrazide linkers.

Keywords: immobilised amylose; phosphorolytic synthesis; maltooligosaccharides; silica gel; silane linkers; hydrazides.

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Figure 2. Synthesis and immobilisation of maltooligosaccharide linkers.

β-Cyclodextrin (β-CD) was cleaved by acid hydrolysis to give linear maltoheptaose,[22] which could be coupled to the ω-unsaturated hydrazides (Fig. 2A).[23] OH-groups were protected with trimethylsilyl chloride (TMS-Cl) and hexamethyl disilazane,[24] and the double bond hydrosilylated[25] using H-Si(OEt$_3$) and bis-cyclopentadienyl platinum chloride.[26] These primers were coupled to porous silica, TMS was removed by treatment with dilute acid in methanol/water to give silica-bonded primers for phosphorolytic amylose synthesis (Fig. 2A).[27]

The $(CH_2)_3$-spacer resulted in inefficient amylose synthesis (not shown), so only the $(CH_2)_6$-, and $(CH_2)_{11}$-spacers were further tested. Among these, $(CH_2)_6$-resulted in a more efficient immobilisation of the maltoheptaose anchor group, giving a maximum spacer density of 72 μmol/g, compared to 22 μmol/g for the 11-C-spacer (Fig. 2B).

2.1. Solid-phase amylose synthesis

Enzymatic synthesis of amylose using potato phosphorylase (EC 2.4.1.1) and glucose-1-phosphate allows generation of amylose polymers with a narrow molecular weight distribution,[28,29] a method which can also be used for the generation of amylose-containing copolymers by heterogeneous phosphorolytic synthesis.[30,31] Similar to anionic polymerisation, a desired molecular weight of the polymer can be defined via the ratio of starter to monomer.[32,28] Since the enzyme requires a maltooligosaccharide starter of at least 4 glucose units in length (Glc$_4$), immobilised maltoheptaose was indeed an efficient starter for polymer synthesis. Enzymatic chain elongation of immobilised maltoheptaose primers afforded silica-bound amylose (Fig. 3A).[32]

Variation of reaction time and amount of enzyme used for synthesis gave an optimal duration of enzymatic synthesis of 15 min, using 0.29 units[33] of potato phosphorylase per μmol of immobilised maltoheptaose starter. The total amount of immobilised polysaccharide after phosphorolytic synthesis was ca. 6%, irrespective of the spacer length (Fig. 3B).[34] The relative increase in amylose content of the modified silica support was higher for the -$(CH_2)_{11}$- spacer, indicating that although fewer immobilised primers were present on the surface, these were better accessible for enzymatic synthesis. Variation of the ratio of monomer (Glc-1-P) to starter (immobilised maltoheptaose) revealed a saturation behaviour, reaching a maximum at a theoretical degree of polymerisation of ca. 100 for -$(CH_2)_6$-, and ca. 250 for the -$(CH_2)_{11}$- spacer (Fig. 3B). This is consistent with a more efficient amylose synthesis from maltoheptaose molecules that are bound to the support via the longer spacer. The saturation behaviour also indicates that an equilibrium of amylose synthesis and removal from the support by a disproportionation reaction of the enzyme[32] was being reached, regardless of the amount of available monomer.

2.2. Characterisation of immobilised amylose

The hydrazide linker had been introduced as a preformed cleavage site to monitor the progress of solid phase amylose synthesis. Under the conditions of synthesis, coupling and deprotection, the hydrazide bond was stable, but synthesised amylose could be cleaved from the support by shaking the material in 0.2 M citrate buffer at pH 4.0 at 60°C for 4 h (Fig. 3A). The supernatant was directly tested by iodine complexation and HPLC (Waters Ultrahydrogel 250, 0.2 M citrate buffer pH 6.0, 0.5 ml/min, room temperature). Synthetic amylose standards[10,35] were used for calibration. Solid-phase synthesised amylose was characterised by a broad molecular weight distribution (Fig. 3C). Reduced accessibility of immobilised primers, leading to variations in the rate of chain elongation on different sites on the support, and beginning disproportionation may account for the observed chain length distribution. High molecular weight of support-generated amylose, and the presence of unreacted primer indicated that only few of the immobilised primer molecules were utilised by the enzyme.

2.3. Chromatography[36]

Amylose-modified silica was suspended in water/0.2% I2/KI solution added to induce formation of the helical amylose–iodine inclusion complex prior to column packing. Water/methanol (95:5, v/v) was used as medium for packing and HPLC. Under these conditions, complex formation ability of amylose was fully preserved, while swelling of the material during column packing was minimised. The observed differences in the elution sequence for complexands of starch and cyclodextrin between base material and amylose-modified silica indicated that bound amylose dominated the separation process (Table 1). In the case of D- and L-menthol, enantioseparation was observed. The chiral separation factor of $\alpha = 1.04$, however, was not sufficient for baseline separation. Note also that known complexands of amylose were not retarded as strongly as expected. Apparently, no inclusion complexes were formed between analytes and immobilised amylose. When amylose phases were removed from the columns and probed with iodine solution, the typical blue colour only became visible after 1–2 min, compared to instantaneous staining with materials that had not been used in HPLC.

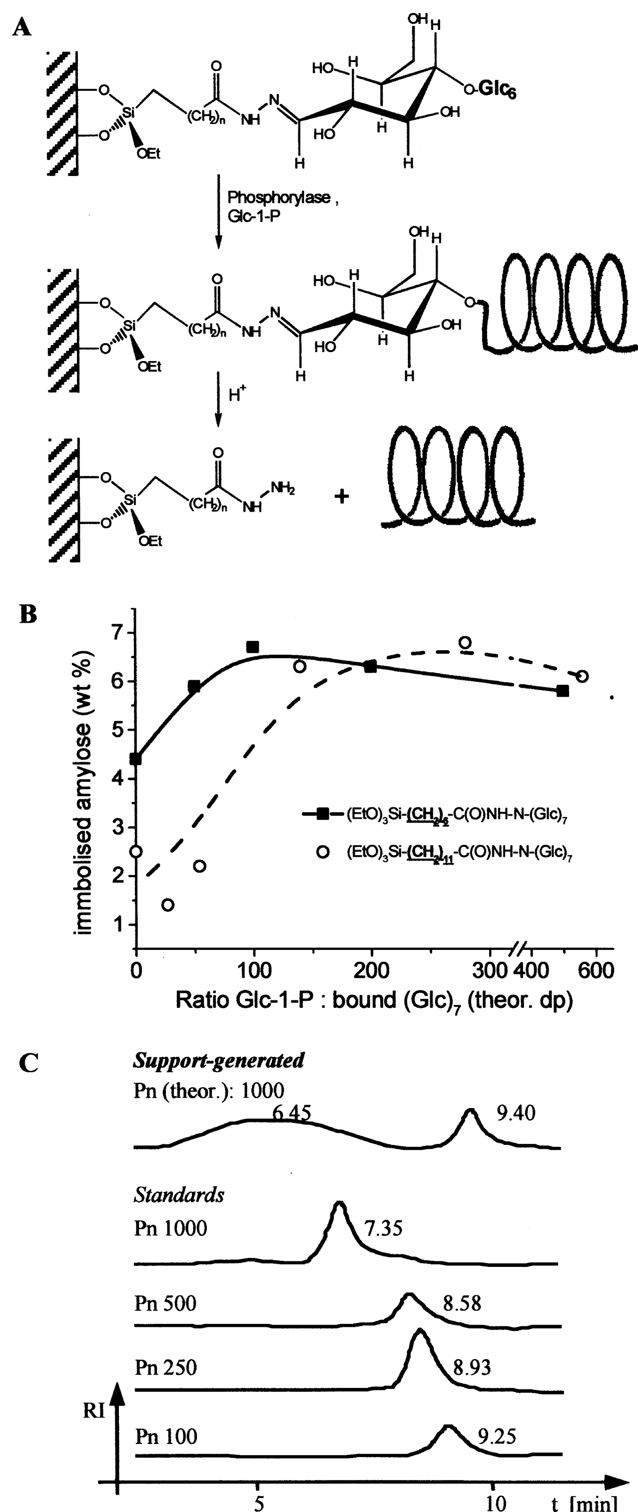

Figure 3. Generation and analysis of support-bound amylose.

Table 1. Chromatographic properties of modified silica phases

Column material	S 250 (base mat.)	S 250-$(CH_2)_{11}$amylose
Amylose (wt%)	–	6.2
Theor. plates	2700	2100
Compound	Capacity factor	
Fenchone	2.16	1.73
D-Menthol	1.94	2.10
L-Menthol	1.94	2.18
2-Hexanone	1.51	1.39
Phenol	1.02	1.09

Thus, the conditions of HPLC column packing and use (>25 bar) induced a conformational change of the amylose, abolishing the low-density, helical V-conformation. Retardation, therefore, was most likely due to interactions on the carbohydrate surface and not complex formation between analyte and amylose helices. Amylose-grafted chromatography materials should thus be useful in low and medium pressure chromatographic applications, where helical inclusion should be the predominant separation mechanism.

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*10-Undecenoylhydrazide **1***: Yield: 97%, mp 86–87°C, ^{1}H NMR ($CDCl_3$): δ 6.92–7.22 (s, 1H, broad, removed with D_2O), 5.54–6.05 (m, 1H), 4.80–5.11 (m, 2H), 3.60–3.93 (s, 2H, broad, removed with D_2O), 1.82–2.26 (m, 6H), 1.10–1.83 (m, 12H). Anal. calcd for $C_{11}H_{22}N_2O$: C, 66.62; H, 11.18; N, 14.13. Found: C, 66.96; H, 11.75; N, 14.11%.
*5-Hexenoylhydrazide **2***: Yield: 81%, mp 46–47°C, ^{1}H NMR ($CDCl_3$): δ 7.25–7.63 (s, 1H, broad, removed with D_2O), 5.51–6.02 (m, 1H), 4.77–5.13 (m, 2H), 3.66–4.06 (s, 2H, broad, removed with D_2O), 1.55–2.31 (m, 6H). Anal. calcd for $C_6H_{12}N_2O$: C, 56.32; H, 9.44; N, 21.86. Found: C, 56.99; H, 10.03; N, 20.85%.
22. *Maltoheptaose **4***: 125 g of β-cyclodextrin (Avebe, Krefeld, Germany) were heated to reflux for 2 h in 500 ml of 0.01 N HCl. After neutralisation, 1 ml 1 M phosphate buffer pH 7.0 was added and the solution stored at 4°C overnight. Unreacted β-cyclodextrin was removed as *p*-xylene complex. Repeated precipitation from water/ethanol and precipitation with acetone gave **4**, R_F 0.19 (*n*-butanol/methanol/water 4:3:3).
23. 5 mmol of maltoheptaose, **4**, were dissolved in 40 ml of dry pyridine; 15 mmol of ω-alkenyl hydrazide were added and the mixture kept at 60°C for 48 h. Solvent was removed and the solid residual washed with ethyl acetate until pyridine-free. *D-Maltoheptaosyl-10-undecenoyl hydrazone **5***: Yield 94%, mp 158–200°C (dec.). Anal. calcd for $C_{52}H_{93}N_2O_{36}\cdot H_2O$: C, 46.60; H, 7.09; N, 2.12. Found: C, 46.77; H, 7.34; N, 2.25%.
*D-Maltoheptaosyl-5-hexenoyl hydrazone **6***: Yield 98%, mp 158–200°C (dec.). Anal. calcd for $C_{48}H_{83}N_2O_{36}\cdot 0.4N_2H_4$: C, 45.15; H, 6.63; N, 3.07. Found: C, 45.13; H, 7.09; N, 3.01%.
*D-Maltoheptaosyl-methacryloyl hydrazone **7***: Yield 85%, mp 170–220°C (dec.). Anal. calcd for $C_{46}H_{79}N_2O_{36}$: C, 44.70; H, 6.44; N, 2.27. Found: C, 44.80; H, 6.87; N, 2.31%.
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Per-O-trimethylsilyl-D-maltoheptaosyl-10-undecenoyl hydrazide: Yield 90%, mp 80–83°C, ^{1}H NMR ($CDCl_3$): δ 4.93–5.32 (m, 9H), 3.19–4.21 (m, broad, 42H), 1.18–2.27 (m, broad, 16H), 0.14 (s, Si-C$\underline{H}_3$). Anal. calcd for $C_{122}H_{277}N_2O_{36}Si_{23}$: C, 48.93; H, 9.32; N, 0.94. Found: C, 48.62; H, 9.54; N, 0.93%.
Per-O-trimethylsilyl-D-maltoheptaosyl-5-hexenoyl hydrazide: Yield 84%, mp 91–94°C, ^{1}H NMR ($CDCl_3$): δ 4.82–5.27 (m, 9H), 3.19–4.21 (m, broad, 42H), 1.82–2.47 (m, broad, 6H), 0.14 (s, Si-C$\underline{H}_3$). Anal. calcd for $C_{117}H_{267}N_2O_{36}Si_{23}$: C, 48.05; H, 9.20; N, 0.96. Found: C, 47.75; H, 9.24; N, 1.30%.
Per-O-trimethylsilyl-D-maltoheptaosyl-methacryloyl hydrazide: Yield 79%, mp 123–128°C, ^{1}H NMR ($CDCl_3$): δ 4.95–5.23 (m, 9H), 3.26–4.18 (m, broad, 42H), 2.1 (2, 2H), 0.14 (s, Si-C$\underline{H}_3$, theor. 484H). Anal. calcd for $C_{115}H_{263}N_2O_{36}Si_{23}$: C, 47.69; H, 9.15; N, 0.97. Found: C, 48.13; H, 9.60; N, 1.05%.
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*Per-O-trimethylsilyl-D-maltoheptaosyl-ω-(triethoxysilyl)-n-undecanoyl hydrazide **8***: mp 80–83°C, ^{1}H NMR ($CDCl_3$): δ 4.93–5.32 (m, 9H), 3.19–4.21 (m, broad, 42H), 3.68 (q, -O-C$\underline{H}_2$), 1.18–2.27 (m, broad, 16H), 1.17 (t, O-CH_2-C$\underline{H}_3$), 0.14 (s, Si-C$\underline{H}_3$). Anal. calcd for $C_{128}H_{293}N_2O_{39}Si_{24}$: C, 48.67; H, 9.35; N, 0.89. Found: C, 48.14; H, 9.16; N, 0.99%.
*Per-O-trimethylsilyl-D-maltoheptaosyl-ω-(triethoxysilyl)-n-hexanoyl hydrazide **9***: mp 91–94°C, ^{1}H NMR ($CDCl_3$): δ 4.82–5.27 (m, 9H), 3.18–4.21 (m, broad, 42H), 3.65 (q, -O-C$\underline{H}_2$), 1.85–2.43 (m, broad, 6H), 1.19 (t, O-CH_2-C$\underline{H}_3$), 0.14 (s, Si-C$\underline{H}_3$); $C_{123}H_{283}O_{39}N_2Si_{24}$: C, 47.83; H, 9.24; N, 0.91. Found: C, 46.56; H, 9.20; N, 1.02.
*Per-O-trimethylsilyl-D-maltoheptaosyl-ω-(triethoxysilyl)-(1-methyl)-n-propionyl hydrazide **10***: mp 123–128°C, ^{1}H NMR ($CDCl_3$): δ 4.95–5.23 (m, 9H), 3.26–4.18 (m, broad, 42H), 3.69 (q, -O-C$\underline{H}_2$), 1.30–2.10 (m, broad, 16H), 1.17 (t, O-CH_2-C$\underline{H}_3$), 0.14 (s, Si-C$\underline{H}_3$). Anal. calcd for $C_{121}H_{279}N_2O_{39}Si_{24}$: C, 47.49; H, 9.19; N, 0.92. Found: C, 46.80; H, 9.23; N, 1.34%.
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