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Synthesis of amylose acetates and amylose sulfates with high structural uniformity

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Abstract

Amylose triacetate (ATA) dissolved in DMSO was partially deacetylated by 1,6-hexamethylendiamine, 1,8-octamethylendiamine, 1,12-dodecylmethylendiamine and 1,2-cyclohexyldiamine (mixture of cis and trans isomers) at 80 °C. The reaction kinetics of the deacetylation were studied. Differences were found in the course of the reaction depending on the type of alkylene diamine (linear or cyclic). The isolated amylose acetates were dissolved in DMF and subsequently sulfated with sulfamic acid. In the course of the sulfation, the acetyl groups acted as protective groups and were completely cleaved after reaction. The amylose acetates and sulfates obtained were studied by ^{13}C NMR spectroscopy and elemental analysis. It could be shown, that the deacetylation of ATA with the described alkylene diamines as well as the subsequent sulfation are highly regioselective. By proceeding this reaction scheme it is possible to synthesize 6-amyloseacetate, 2,6-di-amyloseacetate and 2-amylosesulfate with a high structural uniformity.

Keywords: Amylose triacetate; Alkylene diamine; Regioselective partial deacetylation; Regioselective amylose derivatization; Amylose acetate; Amylose sulfate

1. Introduction

Naturally occurring starch consists of a mixture of the two polysaccharides amylose and amylopectin. Depending on the origin of the starch the percentage composition of both polymers can alter. Because of its wide abundance and polyfunctional character, starch provides a widely used industrial renewable raw material. The chemistry of polysaccharides and the desire to design specific product properties has revealed a number of problems, e.g., regioselectivity of the reaction. Studies on polysaccharide derivatives showed that important parameters for product properties are, for instance, the degree of polymerization (DP), the type of substitution, the total degree of substitution (DS), the distribution of the substituents along the polymer chain and the distribution and site of substituents within the monomer unit (anhydroglucose unit (AGU) for amylose, amylopectin and cellulose). Over the last decades an intensive effort was made to investigate the influence of the site of substitution within the monomer unit on product properties of polysaccharides. In order to obtain polysaccharide derivatives with a preferred site of substitution within the monomer unit (regioselectivity) various preparative strategies were studied. These strategies utilize for instance steric hindrance between the reagent and the polymer (synthesis of 6-*O*-(4-methoxytrityl)-cellulose or 6-*O*-*tert*-butyldimethylsilylcellulose[1,2]), thermodynamic control (sulfation of cellulose with SO_3 in N_2O_4/*N*,*N*-dimethylformamide at various temperatures[3]) or the differing acidity of the hydroxyl groups available for reaction (synthesis of 2-*O*-starch esters[4]).

In the presented work a new pathway to gain amylose derivatives with a high structural uniformity was studied. Based on similar studies on cellulose triacetate,[5] it could be shown, that by partial deacetylation highly acetylated amylose dissolved in DMSO in the presence of water using 1,2-cyclohexyldiamine (CHDA, mixture

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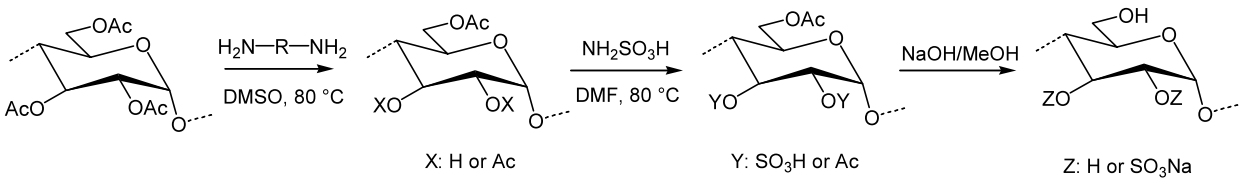

Scheme 1. Reaction scheme for the deacetylation of amylose triacetate, subsequent sulfation of the regenerated hydroxyl groups and cleaving of the acetyl groups with NaOH–MeOH.

of cis and trans isomers), 1,6-hexamethylendiamine (HMDA), 1,8-octamethylendiamine (OMDA) or 1,12-dodecylmethylendiamine (DDMDA) amylose acetates with a high regioselectivity of deacetylation at C-3 or C-2 and C-3 can be obtained. The synthesized 6-amyloseacetate was dissolved in DMF and subsequently sulfated using sulfamic acid. The sulfation reaction led after work up to 2-amylosesulfates with a high structural uniformity (Scheme 1).

2. Experimental

All chemicals were purchased from Sigma and used without pretreatment if not stated otherwise. Amylose used for the experiments was also purchased from Sigma (extracted with 1-butanol from potatoe starch and containing about 10% 1-butanol). According to GPC measurements, the used amylose contained about 11% amylopectin, and the molecular weight corresponded to a degree of polymerization of 13.000.

2.1. Acetylation of amylose[6–9] (1)

For activation prior to reaction 25 g amylose were heated in 1 L water under reflux for 6 h. The mixture of water and partially dissolved amylose was cooled to room temperature (rt) and 500 mL *iso*-PrOH was added. The amylose was filtered off and 1 L *iso*-PrOH was added. The activated amylose was filtered off and dried in vacuum at 40 °C. Depending on the drying time, the amylose contained 5–15% *iso*-PrOH. A mixture of 20 g activated amylose and 330 g Ac_2O were homogenized by applying an Ultra Turrax for 1–2 min.

To the mixture, heated to 90 °C and vigorously stirred, 2.1 g of tetraethylammonium bromide were added. After 15 min, 20 g of a solution of KOH–water (50%, w/w) was added drop wise. Because of the exothermic character of the resulting reaction a water cooler was used while adding the solution of KOH–water. The mixture was stirred at 90 °C for 24 h and dissolved completely. The product was precipitated in warm water. After intensive washing with warm water and *iso*-PrOH, the product was dried in vacuum at 50 °C. ^{13}C NMR (d_6-Me_2SO): δ 19–21 (CH_3-groups of the acetyl groups), 62 (C-6*), 68–72 (C-2*, C-3*, C-5), 74 (C-4), 95 (C-1), 168–172 (carbonyl groups of the acetyl groups), *denotes substitution with acetyl groups; determination of the acetate content by complete alkaline saponification and subsequent titration (Eberstadt-method) resulted in a degree of substitution (DS_{ac}) of 2.95–3.0.

2.2. Deacetylating of amylose triacetate with various diamines (2)

Ten gram of **1** were dissolved in 250 mL Me_2SO at 80 °C under stirring. Sixty milliliter Me_2SO were distilled off under reduced pressure at 95 °C. The vacuum was broken with nitrogen and the reaction flask was cooled to 90 °C. Under vigorous stirring 9.5 g HMDA in 13.7 g water, 9.5 g CHDA (mixture of cis and trans isomers) in 13.7 g water, or 11 g OMDA in 13.7 g water, respectively were added to the solution. After the adding of the water–diamine solution was completed, the mixture was cooled to 80 °C. Samples were taken after differing reaction times (about 30–40 mL each) and poured into glacial AcOH to stop the deacetylation. The products were precipitated in water or *iso*-PrOH (depending on the reaction time and the correlating DS_{ac}) and thoroughly washed.

Deacetylation with DDMDA was carried out by distilling 110 mL Me_2SO under reduced pressure at 95 °C off and adding, after breaking the vacuum with nitrogen, 15.2 g DDMDA dissolved in 13.7 g water and 50 mL Me_2SO (heated to 75 °C) were added. Reaction and work-up were performed as described for HMDA, CHDA and OMDA. ^{13}C NMR (d_6-Me_2SO): δ 19–21 (CH_3-groups of the acetyl groups), 60 (C-6), 62 (C-6*), 68–72 (C-2, C-2*, C-3, C-3*, C-5), 74–80 (C-4), 95–102 (C-1), 168–172 (carbonyl groups of the acetate function), *denotes substitution with acetyl groups; determination of the acetate content by complete alkaline saponification and subsequent titration (Eberstadt-method).

2.3. Sulfation of partially deacetylated acetyl amylose (3)

Nine gram of **2** (moisture content about 4%) were dissolved in 250 mL DMF at 80 °C under stirring. Fifty milliliter solvent were distilled off under reduced pressure at 90 °C. The vacuum was broken with nitrogen and the reaction flask was cooled to 80 °C. 17.3 g sulfamic acid dissolved in 50 mL dry DMF were added

to the solution drop wise. The molar ratio between the reagents resulted in $n_{OH}:n_{NH_2SO_3H} = 1:2$. After 4 h at 80 °C the reaction was stopped by pouring the content of the flask into 500 mL of a 4% (w/w) solution of NaOH in EtOH. The reaction product precipitated and was filtered off. The white product was washed with 100 mL of water to dissolve the inorganic salts. The crude sodium salt of the amylose sulfate was filtered off and dissolved in water for dialysis. After dialysis the product was separated by freeze drying. ^{13}C NMR (D_2O): δ 61 (C-6), 65 (C-6*), 68–72 (C-2, C-2*, C-3, C-3*, C-5), 74–80 (C-4), 96–105 (C-1), *denotes substitution with sulfate groups.

2.4. ^{13}C NMR spectroscopy[10–12]

Amylose acetates were dissolved in d_6-Me_2SO and amylose sulfates were dissolved in D_2O for measurements. The spectra for both polymers were recorded with a Varian UNITY INOVA 500 NMR spectrometer (11.8 T field strength) at 80 °C (d_6-Me_2SO) and 60 °C (D_2O), respectively. To suppress the NOE and to enable quantitative evaluation of the spectra, an inverse gated-decoupling technique was applied. The chemical shifts were referenced to Me_4Si.

2.5. Determination of the DS_{ac} by complete saponification (Eberstadt-method[13])

To 500 mg of powdered amylose acetate 25 mL of water–acetone (1:1, v/v) were given. After 24 h swelling the polymer, 12.5 mL of KOH in EtOH ($c = 1$ M) were added. Complete saponification occurs after 24 h shaking the flask gently at rt. The back titration of the used amount of KOH was performed automatically with a Metrohm 702 SM Titrino and a 0.5 M HCl solution. After completion of the back titration an excess of 2 mL 0.5 M HCl solution was added and after 24 h again titrated with 0.5 M NaOH solution. The acetate content and subsequently the DS_{ac} was calculated according to Eqs. (I) and (II).

$$\text{Bound acetic acid } (\%) = \frac{0.107 \times \text{used KOH (mg)}}{\text{sample weight (mg)}} \quad \text{(I)}$$

$$DS_{ac} = \frac{162 \times \text{AcOH } (\%)}{6000 - 42 \times \text{AcOH } (\%)} \quad \text{(II)}$$

3. Results and discussion

The reaction of amylose triacetate (ATA) with alkylene diamines results in a partial deacetylation of the ATA.

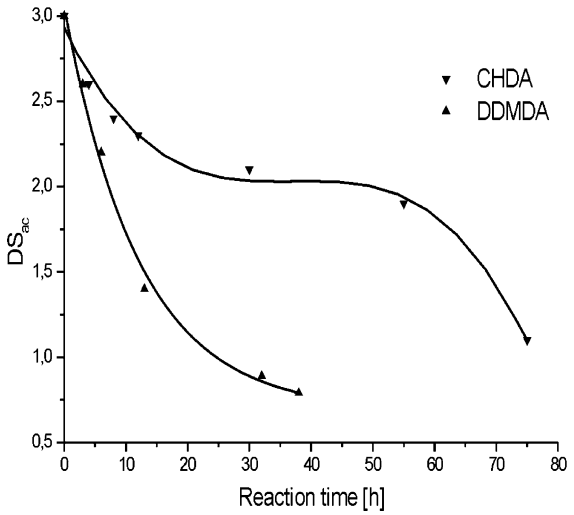

Fig. 1. Reaction kinetics of the deacetylation of amylose triacetate with 1,12-dodecylmethylendiamine and 1,2-cyclohexyldiamine (in DMSO at 80 °C, $n_{AGU}:n_{H_2O} = 1:22$).

The partially deacetylated amylose acetates were subsequently sulfated using sulfamic acid (Scheme 1).

It could be shown that the structure of the alkylene diamines has a deciding influence on the course of the deacetylation and on the products obtained. The reaction kinetics showed significant differences if a linear alkylene diamine (e.g., DDMDA) or a cyclic alkylene diamine (CHDA, mixture of cis and trans isomers) was used for deacetylation (Fig. 1). The kinetics of the deacetylation of ATA with linear alkylene diamines follow a first order reaction. In contrast to these results, during deacetylation with CHDA a plateau in the $t_{reaction}$–DS_{ac}-graph was formed. The plateau is located between $DS_{ac} = 2.1$ and 1.9.

The partially deacetylated amylose acetates were systematically studied with respect to the distribution of substituents within the AGU. In the ^{13}C NMR spectrum (Fig. 2 and Table 1) the quantitative evaluation of the split signals of C-1 and C-6 as well as the quantitative evaluation of the total DS_{ac} (signals of the methyl groups or the carbonyl groups of the acetyl groups) allowed the determination of the distribution within the AGU. Table 2 shows the results of the evaluation of the spectra of some amylose acetates.

The results show that deacetylation is highly regioselective and produces amylose acetates with a high structural uniformity. Depending on the structure of the alkylene diamine, it is possible to obtain 6-amyloseacetate (linear alkylene diamines) or 2,6-di-amyloseacetate (1,2-cyclohexyldiamine). The deacetylation with 1,2-cyclohexyldiamine in particular shows a surprisingly high regioselectivity. These results can be correlated with the data gained from the investigation of the reaction kinetic (Fig. 1), where a plateau is formed

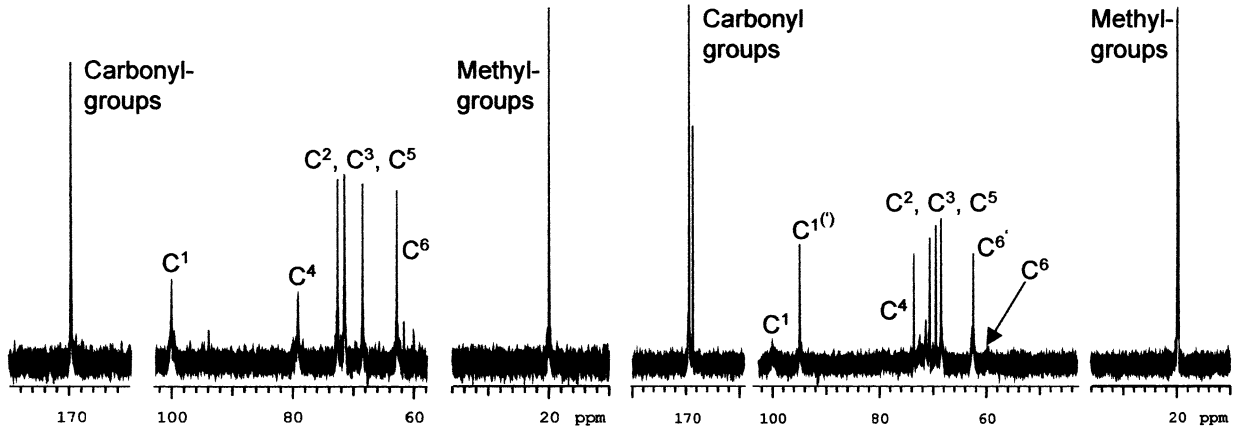

Fig. 2. ^{13}C NMR spectra of amylose acetates; ATA deacetylated with HMDA ($DS_{ac} = 1.1$ [left]) and CHDA ($DS_{ac} = 1.9$ [right]).

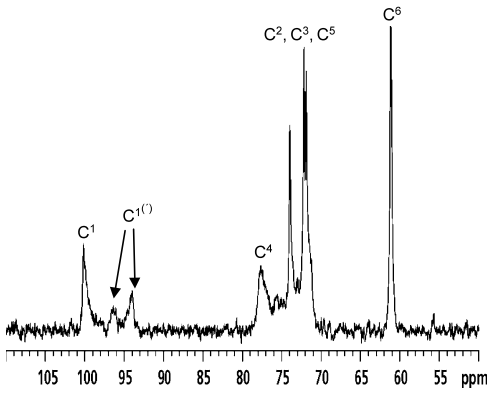

Fig. 3. ^{13}C NMR spectra of a amylose sulfate (ATA deacetylated with DDMDA; $DS_{ac} = 1.9$ and subsequently sulfated with sulfamic acid), $DS_{sulfate} = 0.45$.

between $DS_{ac} = 2.1$ and 1.9 while cleaving the acetyl groups from the ATA with 1,2-cyclohexyldiamine.

While a clear influence of the structure of the alkylene diamines on the regioselectivity of the deacetylation could be proved, no influence of the water content in the reaction mixture or the reaction temperature on the regioselectivity of the deacetylation was found.

It can be assumed, that the conformation of the amylose acetate in solution prevents an attack on the acetyl groups in C-6 and C-2 if a diamine with a rigid, inflexible structure (e.g., CHDA) is used. While 1,2-cyclohexyldiamine is rather inflexible, the linear alkylene diamines are flexible along the alkylene spacer group. For both types of alkylene diamine it was shown, that the acetyl group in C-6 shows the smallest tendency to be cleaved. The reason for that could be a higher thermodynamic stability in combination with a helical structure of the polymer in solution (e.g., all C-6 groups pointing towards the inner side of a helix).

Furthermore, the sulfation of amylose acetates with a DS_{ac} of 1.8 and 1.9 resulted, after work up in a solution of NaOH in EtOH in 2-amylosesulfates (Table 3). Fig. 3 shows the ^{13}C NMR spectra of a 2-amylosesulfate with a triple split of the signal for C-1. It is known, that the signal of C-1 splits if hydroxyl groups on C-2 were functionalized. In case of the 2-amylosesulfate, three peaks appear in total. There were no traces of other functional groups found in ^{13}C NMR that might have caused the triple split. Therefore, we suggest, that all three can be assigned for C-1 either without a sulfate group on C-2 (100 ppm) or for C-1 with a sulfate group on C-2 (94 and 96.5 ppm). Conformational differences of the polymer backbone (e.g., parts of the polymer backbone exist in a supra molecular structure (e.g., helix) other parts exist as e.g., random coil) might be an explanation for the unusual findings.

Table 1
Assignment of the signals in ^{13}C NMR spectra (typical range)

Amylose acetate								
C-atom	1	1 *	2, 2 *, 3, 3 *, 5	4	6	6 *	Methyl	Carbonyl
δ (ppm)	102	96	68–72	74–80	60	62	19–21	168–172
Amylose sulfate								
C-atom	1	1 **	1 **	2, 2 **, 3, 3 **, 5	4	6		
δ (ppm)	100	96.5	94.4	70–75	77.8	61.5		

* Denotes substitution with acetate groups.
** Denotes substitution with sulfate groups.

Table 2
Distribution of acetyl groups within the AGU of partially deacetylated amylose acetates

Diamine used for deacetylation of ATA	DS_{ac} in C-6	DS_{ac} in C-2	DS_{ac} in C-3	DS_{ac} (total)	Reaction time in h
HMDA	1.0	0.4	0.4	1.8	5.5
HMDA	1.0	0.05	0.1	1.15	10
HMDA	0.9	0	0	0.9	13
OMDA	1.0	0.4	0.4	1.8	5.5
OMDA	1.0	0.15	0.15	1.3	13
DDMDA	1.0	0.5	0.4	1.9	7.5
DDMDA	0.95	0.05	0.1	1.1	20.5
CHDA	1.0	0.9	0.2	2.1	30
CHDA	1.0	0.85	0.05	1.9	55
CHDA	1.0	0	0.1	1.1	75

Table 3
Distribution of sulfate groups within the AGU of the sulfated products of the partially deacetylated amylose acetates

	$DS_{sulfate}$ in C-6	$DS_{sulfate}$ in C-2	$DS_{sulfate}$ in C-3	Total $DS_{sulfate}$	
$AmylOSO_3Na$ from $AmylOac_{1.8}$ (HMDA [a])	0	0.62	0	0.62 [b]	0.7 [c]
$AmylOSO_3Na$ from $AmylOac_{1.9}$ (DDMDA [a])	0	0.45	0	0.45 [b]	0.5 [c]

[a] See Table 2.
[b] According to ^{13}C NMR spectroscopy.
[c] According elemental analysis.

4. Conclusion

The results of the partial deacetylation of amylosetriacetate dissolved in DMSO using alkylene diamines at 80 °C open a new strategy for the regioselective functionalization of amylose. While cleaving the acetyl groups, 6-amyloseacetate (using linear alkylene diamines) or 2,6-di-amyloseacetate (using 1,2-cyclohexyldiamine) can be obtained. The surprisingly high regioselectivity of that reaction offers one (in case of the 2,6-di-amyloseacetate) or two (in case of 6-amyloseacetate) hydroxyl groups for further reaction. It could be shown, that the sulfation of amylose acetates with a DS_{ac} of 1.8 and 1.9 with sulfamic acid in solution (DMF at 80 °C) produces amylose sulfates with a $DS_{sulfate}$ of 0.45 and 0.62 in C-2 of the AGU. The amylose acetates used for sulfation carried hydroxyl groups in C-3 of the AGU ($DS_{OH} = 0.6$ for both materials). Although there was a considerable percentage of C-3 free for sulfation, no sulfate groups were found in C-3. Thus, the sulfation of the amylose acetates was found to be highly regioselective. The acetyl groups of the amylose acetates are not cleaved under the sulfation conditions (in DMF, 80 °C, water free)—they were found to be effective protection groups. It is possible to utilize the acetyl groups as protection groups for other chemical modification. Due to the instability of the acetyl groups against saponification, reaction conditions for further modification are limited to non-alkaline media.

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