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Characterisation of the substituent distribution in hydroxypropylated potato amylopectin starch

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Abstract

The distribution of substituents in hydroxypropylated potato amylopectin starch (amylose deficient) modified in a slurry of granular starch (HPPAPg) or in a polymer 'solution' of dissolved starch (HPPAPs), was investigated. The molar substitution (MS) was determined by three different methods: proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, gas-liquid chromatography (GLC) with mass spectrometry, and a colourimetric method. The MS values obtained by ^{1}H NMR spectroscopy were higher than those obtained by GLC–mass spectrometry analysis and colourimetry. The relative ratio of 2-, 3-, and 6-substitution, as well as un-, mono-, and disubstitution in the anhydroglucose unit (AGU) were determined by GLC–mass spectrometry analysis. Results obtained showed no significant difference in molar distribution of hydroxypropyl groups in the AGU between the two derivatives. For analysis of the distribution pattern along the polymer chain, the starch derivatives were hydrolysed by enzymes with different selectivities. Debranching of the polymers indicated that more substituents were located in close vicinity to branching points in HPPAPg than in HPPAPs. Simultaneous α-amylase and amyloglucosidase hydrolysis of HPPAPg liberated more unsubstituted glucose units than the hydrolysis of HPPAPs, indicating a more heterogeneous distribution of substituents in HPPAPg.

Keywords: Hydroxypropylated starch; Potato amylopectin starch; Substituent distribution; GLC–mass spectrometry; Enzymic hydrolysis

1. Introduction

Large quantities of starch are chemically and/or physically modified to obtain desired properties for different applications. One important product group is hydroxypropyl starch that has a wide spectrum of application areas, mainly in food and food-related products due to its storage and freeze/thaw stability [1]. The chemical and functional properties achieved when modifying starch depend on, e.g., reaction conditions, type of substituent, and molar substitution (MS), but may also be affected by the distribution of substituents [2,3]. It is essential to study the relationships between modification procedure, functional properties, and structure, to enable control/direction of the derivatisation process. Therefore, it is of great importance to reliably

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determine structural parameters such as degree of substitution and substituent distribution in starch derivatives.

Several methods for determination of the extent of hydroxypropylation in modified starch have been reported, including a distillation method (Zeisel) [4], colourimetric method (Johnson) [5], and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy [6–9]. The distribution of substituents at the 2-, 3-, and 6-positions in the anhydroglucose unit (AGU) of starch and cellulose derivatives is commonly determined by means of standard methylation analysis [10,11] or reductive cleavage [12,13], followed by separation by gas-liquid chromatography (GLC) and identification by mass spectrometry. Determination of the substituent distribution along the polymer backbone has proven to be more difficult. Methods for analysis of the homo- and heterogeneity of substitution used so far are based on partial degradation of the polymer, either by a random approach with partial acid hydrolysis [14,15] or methanolysis [16], where the results were compared with statistical data calculated for a random substitution pattern, or a selective approach with enzymic degradation [16–19]. The benefits of using enzymic degradations when studying the substitution pattern along the polymer backbone are that (1) different enzymes have different selectivities and (2) the enzyme action is hindered by the substituents. The extent of this hindrance depends on several parameters, such as degree of substitution and type and position of substituents [20].

Hydroxypropyl starch is prepared by a base-catalysed reaction of starch with propylene oxide [1]. The modification process commonly takes place under heterogeneous conditions, i.e., starch is modified with a remaining granular crystalline structure. It may also occur under homogeneous conditions, where the granules are dissolved before reaction. The heterogeneous reaction is believed to enhance the reactivity in the surroundings of an AGU already derivatised, due to a change in polarity or a more 'open' structure with less hydrogen bonds, i.e., a better accessibility. Change in polarity refers to the etherfication of an OH-group and means a local effect somewhere in the polymer chain. At this point the bonding hydrogen pattern is interrupted and the solvation is changed. The result is heterogeneity of the substituent distribution along the polymer chain [15]. Substitution in a homogeneous reaction is expected to take place more uniformly, resulting in a somewhat random substituent distribution [21].

In this work, we report on a study where starch hydrolysing enzymes with different selectivities were used to investigate the homogeneity/heterogeneity of substitution and the location of substituents along the polymer chain in hydroxypropylated potato starch. Moreover, MS values and substituent distribution on a monomer level were determined by GLC–mass spectrometry. Two hydroxypropyl starch samples prepared under different conditions: (i) in a slurry of granular starch (HPPAPg) or (ii) in a polymer 'solution' of dissolved starch (HPPAPs), were investigated. The starch used for hydroxypropylation was genetically modified potato amylopectin starch (PAP). The raw material, devoid of amylose, was therefore more homogeneous than if normal potato starch (20–25% amylose) had been used. The results were discussed with respect to the different modification procedures.

2. Experimental

Substrates.—PAP and hydroxypropylated PAP derivatised in granular slurry (HPPAPg) or solution (HPPAPs), were gifts from Lyckeby Stärkelsen (Kristianstad, Sweden). HPPAPg was prepared by mixing PAP with water and Na_2SO_4. Propylene oxide was added and the reaction was allowed to proceed under alkaline conditions at 40 °C for 48 h. The mixture was neutralised before washing and drying the product. Preparation of HPPAPs was carried out by dissolving PAP in water at 95 °C for 1 h. The remaining part of the modification process was carried on as described above. More detailed information about the production of HPPAP is not allowed to be published by the producer. The water used in all experiments was purified in a Milli-Q system, Millipore (Bedford, MA, USA).

Enzymes.—α-Amylase (EC 3.2.1.1) from *Aspergillus oryzae* (cat. no. E-ANAAM), β-amylase (EC 3.2.1.2) from barley (cat. no. E-BARBP), and amyloglucosidase (EC 3.2.1.3) from *Aspergillus niger* (cat. no. E-AMGPU), were obtained from Megazyme International (Bray, County Wicklow, Ireland). Pullulanase (EC 3.2.1.41) from *Klebsiella pneumoniae* (cat. no. 32-1721) was obtained from ICN Biomedicals, Inc. (Aurora, OH, USA), while isoamylase (EC 3.2.1.68) from *Pseudomonas amylodermosa* (cat. no. EN 102) was from Hayashibara Biochemical Laboratories Inc. (Okayama, Japan).

Permethylation.—Permethylation of the HPPAP samples was carried out according to Ciucanu and Kerek [22]. The samples were dissolved in Me_2SO and the solution ($\approx 1\%$) was treated with powdered NaOH (3 equiv. per OH-group) and MeI (3 equiv. per OH-group) overnight at room temperature with continuous stirring. The methylated products were extracted with CH_2Cl_2 and the organic phase was washed five times with water, dried with $CaCl_2$, and evaporated.

Standard methylation analysis.—Methylated HPPAP (≈ 2 mg) was hydrolysed with 2 M CF_3CO_2H (1 mL) at 120 °C for 3 h in a screw-cap vial housed in a heating and stirring module (no. 18971, Pierce, Rockford, IL, USA). The acid was removed by evaporation, and then the sample was reduced with $NaBD_4$ (0.5 M) in NH_3 (2 M) for 1.5 h at 60 °C. The solution was acidified with HOAc and borate was removed as its methyl ester by mixing and evaporating the reduced product five times with 15% HOAc in MeOH. After evaporation, the sample was acetylated with Ac_2O (200 μL) and pyridine (40 μL) at 90 °C for 3 h. Sodium hydrogen carbonate was added to destroy the excess Ac_2O, the solution was then extracted with CH_2Cl_2, dried with $CaCl_2$, and finally analysed by GLC.

GLC.—GLC was carried out on a GC6000 Vega Series 2 instrument (Carlo Erba) equipped with an on-column injector, a Supelco SPB 5 capillary column (30 m × 0.2 mm), a flame ionisation detector (FID), and a Merck–Hitachi D-2500 integrator. Hydrogen (80 kPa) was used as carrier gas. Temperature program: 60 °C (1 min isothermal); 25 °C/min to 130 °C; then 4 °C/min to 290 °C (30 min isothermal). The molar ratios of the components in the mixture of hydrolysis products were derived from the peak areas in the GLC-FID chromatograms after correction with molar response factors calculated by the effective carbon response concept [23].

GLC–mass spectrometry.—Electron impact ionisation (EI) (70 eV) mass spectra were obtained with a VG analytical VG/70-250S instrument coupled with a Hewlett–Packard HP 5890-A gas chromatograph. For chemical ionisation (CI) mass spectra, ammonia was used as reactant gas.

Determination of β-limit value.—β-Limit values of PAP and HPPAP samples were determined by measuring the amount of maltose released after hydrolysis by β-amylase with the conventional copper sulphate method [24,25]. PAP (5 mg) and HPPAP (15 mg) were gelatinised in NaOH (8 mL, 0.5 M) at 45 °C for 1 h. The pH was adjusted with citric acid (1 M) to 6.0 and the starch solution was incubated with β-amylase (10 U) at 60 °C for 20 h. The β-limit value was calculated as the ratio of the amount of maltose liberated from enzymic hydrolysis and the total content of maltose in the starch sample before hydrolysis.

Enzymic degradation.—Samples of PAP (15 mg) and HPPAP (30 mg) were dissolved in water (4 mL) at 100 °C for 30 min. The pH was adjusted to 5.0 with citric acid (1 M) and the solution was incubated with α-amylase (30 U) at 50 °C for 15 h. Subsequently the pH was adjusted to 4.0 and the solution was incubated for another 15 h with amyloglucosidase (30 U). After deactivation of the enzymes (boiling the solution for 10 min) the amount of glucose liberated was determined using an enzymic/UV glucose test kit from Megazyme (cat. no. R-GLC4).

SEC.—PAP, HPPAP, and their β-limit dextrins were debranched by dissolving the samples (10 mg) in NaOH (1 mL, 0.5 M, 15 min, 40 °C), then the pH was adjusted to 3.5 with citric acid (1 M) and incubated with isoamylase (10 U) for 15 h at 40 °C. The pH was adjusted to 5.5 with NaOH (1 M) and the solution was further incubated with pullulanase (20 U) for a further 15 h. Determination of the molecular weight distributions of

debranched PAP, HPPAP, and their β-limit dextrins was carried out on a size-exclusion chromatographic (SEC) system consisting of a Waters 515 HPLC pump (Waters Corporation, Milford, MA, USA), a SEC column (Microaquagel 20 M, cat. no. 29041) from Chrompack (Nacka, Sweden), and a differential refractive index (DRI) detector (ECR-7512 Erma Cr. Inc., Tokyo, Japan). Elution of the hydrolysis products was performed with NaOH (0.01 M) in NaCl (0.1 M) as mobile phase, at a flow rate of 0.8 mL/min. A degasser from Erma Cr. Inc. (ERC-3112) was used for degassing of the mobile phase. Injection of samples was performed with a Rheodyne injector (no. 7125, Rheodyne, Cotati, CA, USA), equipped with a 100 μL loop (Rheodyne). The chromatographic system was computer controlled by software, JCL6000, from Jones Chromatography (Mid Glamorgan, Wales, UK).

^{1}H NMR spectroscopy.—^{1}H NMR was performed on a 500.13 MHz spectrometer (mod. ARX500, Bruker Fällanden, Switzerland) as described previously [26]. The samples were dissolved in D_2O at 130 °C for 30 min for exchange of hydroxyl protons and then freeze-dried. Finally, the NMR samples were dissolved in D_2O to a concentration of $\approx 1\%$.

Other methods.—Colourimetric determination of MS in HPPAP was performed with ninhydrin according to Johnson [5].

3. Results and discussion

Determination of MS.—MS values of HPPAP samples were determined by three different methods. With ^{1}H NMR spectroscopy, the MS was calculated using the integrated intensities of the signals from the methyl protons in the hydroxypropyl groups ($H(CH_3)$) and the sum of the integrated intensities of the signals from the anomeric protons (H-1), according to Eq. (1). H-1(1→4) denotes the anomeric protons in linear chains, H-1(1→6) the anomeric protons in branching points, H-1(t) the anomeric protons in terminal glucose units, and H-1(HP) the anomeric protons in hydroxypropylated glucose units.

$$\mathrm{MS} = \frac{integral[\mathrm{H(CH_3)}]/3}{integral[\text{H-1(HP)} + \text{H-1(1}\rightarrow\text{4)} + \text{H-1(t)} + \text{H-1(1}\rightarrow\text{6)}]} \quad (1)$$

Fig. 1 shows a ^{1}H NMR spectrum of HPPAPg, where the signal of $H(CH_3)$ appeared at δ 1.15 [8]. The signals of H-1(HP), H-1(1→4), H-1(t), and H-1(1→6) appeared at δ 5.58, 5.35, 5.33, and 4.94, respectively. The chemical shift of H-1(HP) was assigned by two-dimensional homonuclear spectroscopy, while the shifts of the protons in the unsubstituted glucose units have been assigned previously [26]. The MS values derived from ^{1}H NMR were higher than those obtained by standard methylation analysis and the colourimetric

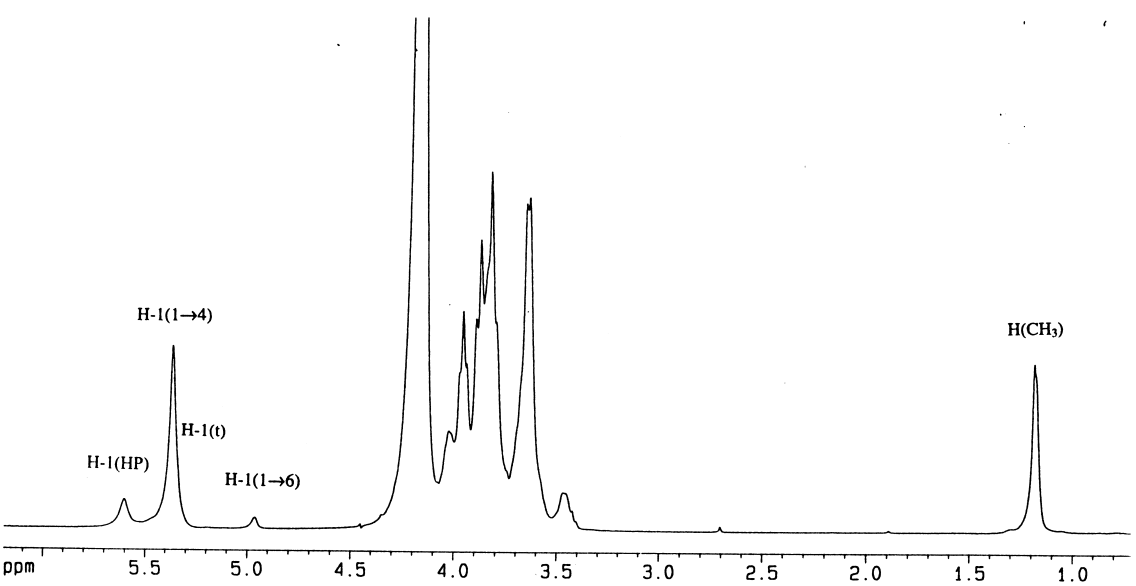


Fig. 1. ^{1}H NMR spectrum of hydroxypropylated potato amylopectin starch prepared in granular slurry.

Table 1
MS values of hydroxypropylated potato amylopectin starch determined by colourimetry, standard methylation analysis, and ^{1}H NMR

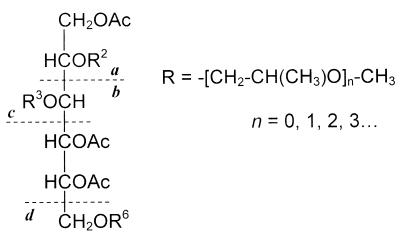
Substrate	MS [a] (colourimetry)	MS [a] (standard methylation)	MS [a] (^{1}H NMR)
HPPAPg	0.16, 0.17	0.15, 0.16	0.21, 0.21
HPPAPs	0.13, 0.14	0.13, 0.13	0.17, 0.17

[a] $n = 2$.

method, which were in good agreement (Table 1). The reason for this significant difference in MS values between the methods is not known. HPPAPg had a slightly higher MS value than HPPAPs.

Monomer analysis.—The distribution of hydroxypropyl groups in the AGU was determined by standard methylation analysis [13]. The HPPAP samples were fully methylated and then completely hydrolysed. The resulting glucose derivatives were converted into their alditol acetate derivatives before characterisation by GLC–mass spectrometry and quantification by GLC. In GLC–mass spectrometry analysis, the number of substituents in the hydrolysate can be derived from CI mass spectra, based on the increase of 58 mass units for every hydroxypropyl group, while the position of substitution can be derived from EI mass spectra. The fragmentation of the glucitol ethers (**1**) follows the principles outlined for partially methylated alditol acetates [27].

Monosubstitution in the AGU at position 2 was determined from the major primary fragment ***a***, $m/z\ 118 + n_2 \times 58$, where n_2 is the number of $-[CH_2-CH(CH_3)O]_n-CH_3$ residues in R^2 (in this case $n_2 = 1$), in combination with the primary fragment ***b***, m/z 233. In addition, consecutive losses of acetic acid from ***b*** (***b***-120) gave a strong secondary fragment (m/z 113). Similar, monosubstitution at position 3 was deduced from the occurrence of the primary fragments ***a*** (m/z 118) in combination with ***b*** ($m/z\ 233 + n_3 \times 58$, where $n_3 = 1$) and ***c*** ($m/z\ \ 162 + n_3 \times 58,\ \ n_3 = 1$). The secondary fragmentation of ***b*** with losses of acetic acid and keten (***b***-102) was also of diagnostic value. Determination of substitution at position 6 was made from the combination of the primary fragments ***a*** (m/z 118), ***b*** (m/z 291), and ***c*** (m/z 162), but also of importance was the secondary fragments of ***b*** (***b***-120; consecutive losses of acetic acid) and ***c*** (***c***-60; loss of acetic acid). Disubstituted derivatives (2,3-, 2,6-, and 2,3-substituted) were determined following the same procedure as described above.



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When identifying the components in the mixture it was also valuable to consider the regularities that exist in the elution order in GLC, which depend on the position of substitution in the AGU; monomers substituted at position 3 elute before those substituted at position 2 and finally monomers substituted at position 6 elute. The elution order of disubstituted components follows the corresponding combinations; 2,3 before 3,6, followed by 2,6.

The molar ratios of the components in the mixture of partially methylated alditol acetates (Table 2), calculated from the peak areas in the chromatograms, showed that substitution of hydroxypropyl groups occurred primarily at position 2 in both samples, which is the expected result for a base-catalysed etherfication. The higher reactivity of HO-2 (a secondary hydroxyl) over HO-6 (a primary hydroxyl) is not fully understood, but one possible reason could be the relative high acidity of HO-2 due to the proximity to the anomeric centre [28]. This argument is supported by the fact that the regioselectivity of 2-*O*-substitution is strongly enhanced with decreasing amount of base and therefore higher for oxirane addition than for Williamson etherfication processes. However, there must

be an additional effect of the α-configuration (e.g., a neighbour participation), since this selectivity is much less pronounced for β-linked cellulose. For HPPAPg, HO-3 and HO-6 were found to possess the same reactivity, while in HPPAPs, HO-3 showed slightly higher reactivity than HO-6. The substitution pattern on a monomer level in the two HPPAP samples was similar, which indicates that the substituent distribution in the AGU is unaffected by the reaction conditions. Similar results have been reported previously in investigations of other starch derivatives [18].

Enzymic degradation.—β-Amylase is an exo-enzyme that hydrolyses every second α-(1→4) D-glucosidic linkage from the non-reducing end, but stops on average two glucose units before a branching point. This action results in liberation of β-maltose and a remaining β-limit dextrin [29]. When comparing the β-limit values of the unmodified and modified samples (Table 3) it was shown that β-amylase action was hindered by the hydroxypropyl groups in the exterior chains (i.e., those chains located outside the branching points) of the HPPAP samples, since hydrolysis of these samples liberated less maltose compared with hydrolysis of unmodified PAP. Thus, there are hydroxypropyl groups located outside the branching points in both derivatives. HPPAPg and HPPAPs had almost the same β-limit value, indicating that the average distance from the non-reducing end to the first hydroxypropyl group was similar in these two samples.

Table 2
Distribution of substituents in hydroxypropylated potato amylopectin starch obtained by standard methylation analysis

Position substituted	HPPAPg (mol%)	HPPAPs (mol%)
-	84.7	87.5
2	12.1	9.8
3	1.1	1.3
6	1.1	0.8
2,3	0.7	0.4
2,6	0.3	0.2
3,6	n.d. [a]	n.d. [a]
2,3,6	n.d. [a]	n.d. [a]
Unsubstituted	84.7	87.5
ΣMonosubstituted	14.3	11.9
ΣDisubstituted	1.0	0.6
MS	0.16	0.13

[a] n.d. = not determined.

Table 3
β-Limit values and amount of glucose liberated from enzymic hydrolysis of unmodified and hydroxypropylated potato amylopectin starch

Substrate	β-Limit value (%) [a]	Liberated glucose (%) [a,b]
PAP	51.1 (1.1)	95 (3.6)
HPPAPg	10.4 (2.6)	70.6 (4.1)
HPPAPs	11.3 (2.4)	58.1 (4.5)

[a] Mean values, $n = 3$, standard deviations are given within brackets.

[b] The relative amount of liberated glucose is referred to the unsubstituted AGUs.

Isoamylase and pullulanase are debranching enzymes that catalyse the hydrolysis of α-(1→6) D-glucosidic linkages in branched polysaccharides [29]. Determination of the size distribution of PAP and HPPAP debranched by isoamylase and pullulanase was performed with SEC-DRI. PAP was completely debranched, i.e., the hydrolysate consisted of only linear chains. Debranching of HPPAPs showed a broad size distribution of larger hydrolysis products compared with PAP, whereas debranching of HPPAPg resulted in products having even higher molecular weights. As expected from these results, debranching of the β-limit dextrins of PAP and HPPAP showed that β-limit dextrin of HPPAPg was more resistant to hydrolysis by isoamylase and pullulanase than was the dextrin of HPPAPs, while the β-limit dextrin of PAP was completely debranched. Obviously, the substituents hinder the action of isoamylase and pullulanase, since the enzymes are incapable of debranching the hydroxypropyl starches completely. Therefore, it is likely that there are hydroxypropyl groups located in the close vicinity to branching points. HPPAPg and its β-limit dextrin were debranched to a lesser extent compared with HPPAPs, indicating that there are more substituent groups located near the branching points in HPPAPg. This could be explained by the fact that starch is a semi-crystalline polymer in which the amylopectin according to the cluster model [30] consists of alternating crystalline and amorphous regions. The crystalline domains

are formed by double-helical arrangements of the outer parts of the side-chains of amylopectin, whereas the regions in the vicinity of the branching points are responsible for the amorphous parts [31]. Several authors have reported on a higher reactivity in the amorphous parts of amylopectin compared with the crystalline areas; thus substitution occurs preferentially in the amorphous zones [17,19,32,33]. Consequently, HPPAPg that was modified with remaining crystalline structure showed a higher reactivity around the branching points compared with HPPAPs in which the crystallinity was destroyed before modification and thus had a more uniform reactivity in the different parts of the molecule [34]. These observations indicate a more heterogeneous substitution pattern in HPPAPg compared with HPPAPs.

α-Amylase is an endo-enzyme that catalyses the random hydrolysis of α-(1→4) D-glucosidic linkages in polysaccharides containing three or more α-(1→4) linked D-glucose units, resulting in the production of glucose and oligosaccharides containing two to seven glucose residues [29]. Amyloglucosidase acts in an exo manner and catalyses the successive hydrolysis of terminal α-(1→4) linked α-D-glucose residues from the non-reducing end, with release of β-glucose. The enzyme also has the capacity to cleave α-(1→6) D-glucosidic linkages that are surrounded by glucose units linked by α-(1→4) linkages [29]. Simultaneous α-amylase and amyloglucosidase hydrolysis of PAP resulted in complete conversion into glucose, whereas 71% of HPPAPg and 58% of HPPAPs were hydrolysed into glucose (Table 3). The amount of glucose liberated was determined and referred to the unsubstituted AGUs present in the HPPAP samples. Obviously, the enzymes were unable to degrade the derivatives completely due to the substituents. For a given MS, the amount of glucose liberated by enzymic hydrolysis under defined conditions should increase with increasing heterogeneity of substitution in the polymer chain, since the average length of unsubstituted sequences is longer when the substituents are more clustered [35]. As a consequence, the enzymic hydrolysis of heterogeneously modified derivatives is expected to liberate more glucose than the hydrolysis of homogeneously modified derivatives. The results obtained here are in agreement with this theory, since the heterogeneously modified HPPAPg liberated more glucose than did the homogeneously modified HPPAPs. Similar results have been reported previously for other starch derivatives [17,18].

Enzymic (α-amylase) digestibility of hydroxypropylated starch was examined by Leegwater [28], who concluded that hydroxypropylated glucose units block the cleavage of adjacent glucosidic bonds and that the reducing power in the enzyme hydrolysate decreases with increasing substitution. Several other authors report on similar results from enzymic digestion of hydroxypropylated starch [36,37]. However, no more detailed information about the effects of hydroxypropyl groups on the action of different enzymes is available. Recently, Kavitha et al. [19] presented an investigation in which several different starch hydrolysing enzymes were used to determine the location of hydroxypropyl groups in modified potato starch. The results showed that there were regions in both amylose and amylopectin where the substituent groups occurred in clusters.

Exterior and interior chain lengths.—Simultaneous information about MS and average chain length (CL) in the unmodified and modified starch samples were achieved by ^{1}H NMR spectroscopy. The average CL was calculated according to Eq. (2).

$$\text{Average CL} = \textit{integral}\,\frac{[\text{H-1}(1\rightarrow4)+\text{H-1(t)}+\text{H-1}(1\rightarrow6)]}{[\text{H-1}(1\rightarrow6)]} \quad (2)$$

With knowledge of the β-limit value, it was also possible to calculate the average length of the exterior chains (ECL) according to Eq. (3) [38]. The addition of 2 is derived from the assumption that ECL in a β-limit dextrin of unmodified starch is on average two glucose units.

$$\text{ECL} = \text{CL} \times (\%\ \beta\text{-limit}/100) + 2 \quad (3)$$

Furthermore, the average length of the interior chains (ICL) was calculated according to Eq. (4) [38]. (Subtraction of 1 due to the AGU with both an α-(1→4) and α-(1→6) linkage;

Table 4
Average chain length values of unmodified and hydroxypropylated potato amylopectin starch and their β-limit dextrins determined by ^{1}H NMR spectroscopy

Substrate	CL [a] (^{1}H NMR)	CL [a] in β-limit dextrin (^{1}H NMR)	ECL [b]	ECL [b] in β-limit dextrin	ICL [c] (Eq. (4))
PAP	25.6	11.7	15.1	2 [d]	9.5
HPPAPg	25.5	20.7	15.1 [e]	10.2	9.5 [e]
HPPAPs	24.3	19.9	15.1 [e]	9.4	9.5[e]

[a] CL = average chain length.
[b] ECL = average exterior chain length.
[c] ICL = average interior chain length.
[d] ECL in β-limit dextrin of unmodified starch is assumed to be two.
[e] ECL and ICL in HPPAP are approximated to be equal to the ECL and ICL in native PAP.

this monomer is by definition not included in neither exterior nor interior chains).

$$ICL = CL - ECL - 1 \quad (4)$$

For calculation of ICL in a β-limit dextrin of unmodified starch ($ICL_{\beta\text{-limit}}$), Eq. (4) could be simplified if assuming that ECL in the β-limit dextrin is on average two glucose units:

$$ICL_{\beta\text{-limit}} = CL_{\beta\text{-limit}} - 3 \quad (5)$$

The results from ^{1}H NMR analysis of PAP, HPPAP, and their β-limit dextrins (Table 4) showed that PAP and HPPAP had the same CL, which was expected since the HPPAP samples originate from the PAP sample (i.e., hydroxypropylation of potato amylopectin starch does not change the CL). The CLs in the β-limit dextrins of the HPPAP samples were longer in comparison with the dextrin of PAP, which confirms the result obtained previously (Section 3.3), i.e., β-amylase is hindered by the hydroxypropyl groups in the exterior chains. ECL in PAP was calculated according to Eq. (3), but this equation is not valid for calculations of ECL in HPPAP due to the assumption that ECL in β-limit dextrin is two glucose units, which is incorrect in the case of hydroxypropyl starch. Therefore, it was assumed that ECL and ICL were approximately the same in unmodified and modified starch, since modification should not affect the length of the chains. This assumption made it possible to calculate ECL in the β-limit dextrins of HPPAP with Eq. (4). According to the results obtained, ECL in the HPPAP samples was on average five glucose units shorter after β-amylase hydrolysis. This means, as a rough approximation, that the enzyme hydrolyses on average five glucose units of the exterior chains, starting from the non-reducing end. It was therefore assumed that substitution of the exterior chains occurs on average from the seventh AGU. There was no significant difference in ECL between the two HPPAP samples, indicating that substitution of the outer parts of the exterior chains was somewhat independent of the reaction conditions.

ICL in the β-limit dextrin of PAP was calculated to be 9.5 (Table 4) with Eq. (4), while calculation according to Eq. (5) resulted in an ICL of 8.7. The values obtained were in agreement, thus any of the equations can be used.

4. Conclusions

Hydroxypropylated potato amylopectin starch modified in granular slurry or solution has been investigated with respect to the distribution of substituents in the polymer. Standard methylation/GLC–mass spectrometry analysis showed that the distribution of substituents in the AGU was independent of the modification conditions, and also that hydroxypropylation occurred primarily at HO-2. From enzymic hydrolysis of the derivatives it was concluded that the hydroxypropyl groups hindered the action of starch hydrolysing enzymes. For debranching enzymes, this hindrance was more pronounced when hydrolysing HPPAPg compared with HPPAPs, which indicated that there were more substituent groups located near the branching points in the former substrate. Further, HPPAPg was degraded to a higher extent by

simultaneous exo- and endo-enzymic hydrolysis and thus had a more heterogeneous substituent distribution compared with HPPAPs. The results are taken as evidence that the modification conditions affect the distribution of hydroxypropyl groups along the polymer chain. From ^{1}H MNR analysis of ECL before and after β-amylase hydrolysis, it was assumed that hydroxypropylation of the exterior chains occurred on average from the seventh glucose unit from the non-reducing end.

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References

[1] O.B. Wurzburg, *Modified Starches; Properties and Uses*, 3rd ed., CRC Press, New Jersey, 1986, pp. 89–96.
[2] S.I. Takahashi, T. Fujimoto, T. Miyamoto, H. Inagaki, *J. Polym. Sci.*, 25 (1987) 987–994.
[3] I. Nehls, B. Philipp, W. Wagenknecht, D. Klemm, M. Schnabelrauch, A. Stein, T. Heinze, *Das Pap.*, 44 (1990) 633–640.
[4] H.J. Lortz, *Anal. Chem.*, 28 (1956) 892–899.
[5] D.P. Johnson, *Anal. Chem.*, 41 (1969) 859–860.
[6] H. Stahl, R.P. McNaught, *Cereal Chem.*, 47 (1970) 345–350.
[7] R.A. de Graaf, G. Lammers, L.P.B.M. Janssen, A.A.C.M. Beenackers, *Starch/Stärke*, 47 (1995) 469–475.
[8] A. Xu, P.A. Seib, *J. Cereal Sci.*, 25 (1997) 17–26.
[9] I. Nehls, W. Wagenknecht, B. Philipp, O. Stscherbina, *Prog. Polym. Sci.*, 19 (1994) 29–78.
[10] O. Larm, K. Larsson, O. Theander, *Starch/Stärke*, 33 (1981) 240–244.
[11] B. Lindberg, U. Lindquist, O. Stenberg, *Carbohydr. Res.*, 170 (1987) 207–214.
[12] A.J. d'Ambra, M.J. Rice, S.G. Zeller, P.R. Gruber, G.R. Gray, *Carbohydr. Res.*, 177 (1988) 111–116.
[13] P. Mischnick, *Carbohydr. Res.*, 192 (1989) 233–241.
[14] P.W. Arisz, H.J.J. Kauw, J.J. Boon, *Carbohydr. Res.*, 271 (1995) 1–14.
[15] P. Mischnick, G. Kuhn, *Carbohydr. Res.*, 290 (1996) 199–207.
[16] Y.E.M. van der Burgt, J. Bergsma, I.P. Bleeker, P.J.H.C. Mijland, A. van der Kerk-van Hoof, J.P. Kamerling, J.F.G. Vliegenthart, *Carbohydr. Res.*, 312 (1998) 201–208.
[17] P. Steeneken, A. Woortman, *Carbohydr. Res.*, 258 (1994) 207–221.
[18] O. Wilke, P. Mischnick, *Starch/Stärke*, 49 (1997) 453–458.
[19] R. Kavitha, J.N. BeMiller, *Carbohydr. Polym.*, 37 (1998) 115–121.
[20] L.J. Filer, *Nutr. Rev.*, 29 (1971) 55–59.
[21] G. Fleche, in G.M.A. van Beynum, J.A. Roels (Eds.), *Starch Conversion Technology*, Marcel Dekker, New York, 1985, pp. 73–99.
[22] I. Ciucanu, F. Kerek, *Carbohydr. Res.*, 131 (1984) 209–217.
[23] D.P. Sweet, R.H. Shapiro, P. Albersheim, *Carbohydr. Res.*, 40 (1975) 217–225.
[24] M. Somogyi, *J. Biol. Chem.*, 195 (1952) 19–23.
[25] N. Nelson, *J. Biol. Chem.*, 153 (1944) 375–380.
[26] G.S. Nilsson, K.-E. Bergquist, U. Nilsson, L. Gorton, *Starch/Stärke*, 48 (1996) 352–357.
[27] H. Björndal, B. Lindberg, S. Svensson, *Carbohydr. Res.*, 5 (1967) 433–440.
[28] D.C. Leegwater, *Starch/Stärke*, 24 (1971) 11–15.
[29] A. Guilbot, C. Mercier, in G. Aspinall (Ed.), *The Polysaccharides*, Vol. 3, Academic Press, New York, 1985, pp. 210–273.
[30] D. French, *J. Jpn. Soc. Starch Sci.*, 19 (1972) 8–26.
[31] J.P. Robin, C. Mercier, R. Charbonnière, A. Guilbot, *Cereal Chem.*, 51 (1974) 389–406.
[32] P.A.M. Steeneken, E. Smith, *Carbohydr. Res.*, 209 (1991) 239–249.
[33] L.F. Hood, C. Mercier, *Carbohydr. Res.*, 61 (1978) 53–66.
[34] P.A.M. Steeneken, A.C. Tas, A.J.J. Woortman, P. Sanders, *Spec. Publ.-R. Soc. Chem.*, 205 (1997) 153–162.
[35] R.A. Gelman, *J. Appl. Pol. Sci.*, 27 (1982) 2957–2964.
[36] L.F. Hood, V.G. Arneson, *Cereal Chem.*, 53 (1976) 282–290.
[37] M. Wootton, M.A. Chandry, *Starch/Stärke*, 31 (1979) 224–228.
[38] D.J. Manners, *Carbohydr. Polym.*, 11 (1989) 87–112.