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Carbohydrate Polymers 65 (2006) 529–534
Carbohydrate Polymers
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The azidation of starch ☆

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Received 29 December 2005; accepted 8 February 2006
Available online 12 May 2006

Abstract

Starch is an inexpensive commodity that has been used for non-food purposes for many years. Some of these uses include cross-linked starches that are synthesized with a variety of multifunctional reagents. One unexplored possibility is the use of azides for cross-linking. To this end, azide derivatives of different starches have been synthesized, including the first reported synthesis of 6-deoxy-6-azido-amylopectin. Lithium salts, which were found to not be essential for the dissolution of starch in the reaction, were replaced with sodium azide. The time for this derivatization reaction to reach completion was determined to be 1 h. *N*,*N*-dimethylacetamide was also found to be a suitable solvent. Initial experiments suggest that the azide derivative does cross-link starch when activated by heat.
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Keywords: Starch; Azide; Modified starch; Azidation; Amylose; Amylopectin

1. Introduction

Starch, a surplus food commodity, has been studied for non-food applications for many years (Whistler, BeMiller, & Paschall, 1984). Due to their availability and cost, modified starches have also been used in many non-food applications. For example, cationic starches and starch phosphate esters have been used as adhesives and coatings in papermaking, as flocculants in water, and as sizers in textiles. Dextrinized starches have been used for adhesive purposes. Also, acid-modified, oxidized, hydroxyalkyl, and esterified starches have been used in the manufacturing of paper, paperboard, and gypsum boards as well as in the textile industry. Moreover, there is interest in esters for slow release of pesticides (McGuire, Wing, & Doane, 1981). In addition, cross-linked starches are used in antiperspirants and in applications where insoluble granular starches are needed.

Cross-linking of starch has been achieved with a variety of multifunctional reagents (Whistler et al., 1984). One unexplored possibility is with the use of azides. The synthesis and use of azides in organic chemistry are well known (Scriven & Turnbull, 1988), and arylazides have been used to cross-link polymers in microlithography (Willson, 1994). Preparation of the azido group in carbohydrate chemistry has also been studied. Specifically, primary hydroxyl groups in monosaccharides (Blanco, Fernández, Gadelle, & Defaye, 1997; Castro, Chapleur, Gross, & Selve, 1972), oligosaccharides (Blanco et al., 1997) including cyclodextrins (Boger, Corcoran, & Lehn, 1978), and polysaccharides (Cimecioglu, Ball, Huang, & Kaplan, 1997; Cimecioglu, Ball, Kaplan, & Huang, 1994) have been converted into azides. The azide derivative is desirable since it can be mildly and selectively reduced to the corresponding amino saccharide via the Staudinger reaction (Cimecioglu

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name. USDA implies no approval of the product to the exclusion of others that may also be suitable.

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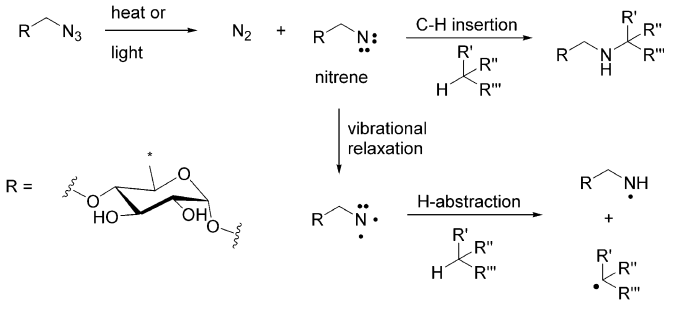
0144-8617/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2006.02.009



Scheme 1. Azide decomposition and subsequent reactions.

et al., 1994). While azido saccharides are usually synthesized for the amino group, the chemistry of azides allows it to be used for cross-linking purposes. Generally, the azide can be thermolyzed or photolyzed to produce a nitrene and nitrogen gas (Scheme 1). The nitrene, depending on whether it is in a singlet or triplet state, can undergo C–H insertion or H-abstraction (Scriven & Turnbull, 1988). The result of a C–H insertion is a cross-linked product. H-abstraction results in radicals that can also lead to cross-linked products. C–H insertion and H-abstraction are believed to occur with arylazides in microlithography, in fact, increase in the molecular weight of polymers resulting from azide cross-linking has been observed (Willson, 1994). Cross-linking reagents are typically multifunctional low molecular weight compounds that are incorporated into the polymer. In this case, the cross-linkable functional group is directly synthesized onto the backbone of the polymer. This approach to cross-linking can be applied to any primary hydroxyl-containing polymer and might lead to processing of biopolymers with previously incompatible polymers.

Cimecioglu et al. (1997) have recently discovered that amylose and pullulan can be derivatized to the corresponding 6-azido-6-deoxy polysaccharide in one step. Since amylose and pullulan are expensive, extending this reaction to relatively inexpensive materials is desirable. In this report, the one pot azidation reaction was further studied and applied to different starches. The reaction was expanded to include the use of the more common NaN_3 instead of LiN_3. Also, this reaction was found to proceed in DMAc as well as DMF, which is potentially useful for derivatizing cellulose (McCormick, 1981). Rate studies also suggest that the reaction is complete under the reaction conditions within 1 h.

2. Experimental

2.1. Materials

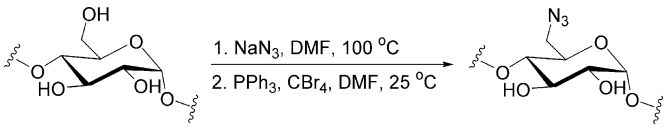
Amylose (Type III and BioChemika) and amylopectin were obtained from Sigma-Aldrich (St. Louis, MO). Native potato starch was Potato Starch Superior from Emsland (Emlichheim, Germany), and native wheat starch was Midsol 50 from MGP Ingredients (Atchison, KS). Waxy cornstarch was from Penford (Englewood, CO), and high amylose corn starch (HACS) was from National Starch (Bridgewater, NJ). Pregelatinized potato starch was from Penford as PenCook 10, and pregelatinized wheat starch was from MGP Ingredients. Sodium azide (NaN_3) and methanol (MeOH) were from Fisher Scientific (Hampton, NH). Triphenylphosphine (PPh_3) and carbon tetrabromide (CBr_4) were from Sigma–Aldrich. Ethanol (EtOH) was from Pharmco Products (Brookfield, CT) and Aaper Alcohol (Shelbyville, KY). Anhydrous *N*,*N*-dimethylformamide (DMF), *N*,*N*-dimethylacetamide (DMAc), anhydrous lithium bromide (LiBr), anhydrous lithium chloride (LiCl), and anhydrous dimethylsulfoxide (DMSO) were from Sigma–Aldrich. Ultra high purity nitrogen (N_2) was from Praxair (Danbury, CT). All chemicals except DMF were used as received. DMF was distilled from CaO prior to use (Gordon & Ford, 1972).

2.2. Pregelatinized HACS

High amylose corn starch at 12.4% moisture was gelatinized at 8 wt% solids in a Parr 4521M pressure reactor controlled with a Parr 4843 temperature controller (Moline, IL) reactor. Total cooking time was 50 min of which 20 min was at temperatures exceeding 130 °C. The sample was then stored overnight at 4 °C, and the resulting gel was homogenized with a high shear mixer for 1 min. Next, water in the homogenate was replaced with EtOH in a series of solvent exchange washes. The successive washes were 40%, 60%, 80%, 95%, and 100% (three times) EtOH, respectively. Each wash was allowed to equilibrate for at least 4 h. The gelatinized HACS was then oven dried at 105 °C. The resulting cake was ground with a mortar and pestle and subsequently used in the azidation reaction.

2.3. Azidation procedure

A typical azidation reaction is carried out as shown in Scheme 2. First, 162 mg (1 mmol) starch, 650 mg (10 mmol) of sodium azide, and 5 mL distilled DMF were added to a N_2-flushed oven-dried 3-neck round bottomed flask equipped with a stir bar and a thermometer. The reaction solution was subsequently heated to 100 °C with a constant N_2 flush for 1 h. The reaction was then cooled in an ice water bath before 524 mg (2 mmol) of PPh_3 was added. Next, 663 mg (2 mmol) CBr_4 was dissolved in 2 mL of distilled DMF. The CBr_4 solution was then slowly added to the 3-neck round bottomed flask. The reaction was removed from the ice water bath and allowed to warm up to room temperature overnight with positive N_2 pres-



Scheme 2. Azidation reaction.

sure. The reaction is initially an orange color that turns tan or brown. Methanol (1 mL) was added to quench the reaction, and the starch was precipitated by addition of ethanol (200 mL). The precipitate was recovered by filtration through a Millipore nylon 0.45 μm pore membrane. The filter cake was then washed with 150 mL of a 7:3 ethanol/water solution followed by 100 mL ethanol. The filter cake was then allowed to air dry. This procedure is referred to in the text as two equivalents of reagents to starch. When one equivalent of reagents to starch is mentioned, one equivalent of PPh_3 and CBr_4, and five equivalents of sodium azide were used.

Minor changes were made to the typical procedure depending on the specific reaction conditions. When DMAc was used instead of DMF, the volume of DMAc to dissolve CBr_4 was doubled. In reactions that used LiBr or LiCl, it was added first to the reaction at the same equivalence as sodium azide. Solubility and reactivity differences between Type III amylose and BioChemika amylose were evident, with Type III amylose being more amenable to conversion. This discrepancy in reactivity is attributed to the different isolation procedures for the amylose. Also, due to the waxy nature of amylopectin the filtration can proceed slowly. One solution was to filter small volumes (20 mL) at a time. The amylopectin also tends to stick on the nylon membrane. Separation can be achieved by first heating the membrane in distilled water, then separating the starch from the membrane, followed by freeze-drying. This processing did not affect yields.

For larger scale reactions, filtration could be very time consuming, so a method of isolating the starch by centrifugation was devised. First, the precipitated reaction solution was divided equally into centrifuge bottles. These solutions were then stirred for a few minutes to disperse the reacted starch. Next, the bottles were centrifuged at 3500 rpm for 15 min and then the supernatant was decanted. This process was repeated two times with EtOH, three times with 7:3 EtOH/water, and one more time with EtOH. After the last decanting, the washed starch could be transferred onto a Millipore filter with a nylon membrane to remove the remaining liquid, or it could be crushed and dried overnight at 60 °C under vacuum. Better recovery of starch was observed on larger scales. Finally, for larger scale reactions, two equivalents of reagents were not needed to achieve full conversion of the starch.

2.4. Analyses

^1H (400 MHz) and ^{13}C NMR spectra (100 MHz) were obtained on a Bruker ARX 400 spectrometer in DMSO-d_6 solutions at 20–80 mg/mL referenced to TMS (0.00 ppm). Reaction products were dissolved for NMR analyses by alternating heat (<80 °C) and agitation with a vortex mixer. Percent conversions were determined using ^{13}C NMR by integrating the C-6 primary carbon at 60 ppm (unreacted starch) and 51 ppm (azide product). There was no evidence from NMR of azide substitution other than at the C-6 carbon. IR spectra were obtained on a Perkin-Elmer System 2000 equipped with an attenuated total reflectance (ATR) based DuraSamplIR probe from SensIR Technologies (Danbury, CT). Elemental Analysis was by Schwarzkopf Microanalytical Laboratory (Woodside, NY). Thermogravimetric analysis was on a TGA 2950 from TA Instruments (New Castle, DE). Isothermal experiments were performed by first heating at 20 °C/min to 160 °C, then holding the temperature constant for 6 h, and finally heating to 1000 °C at 20 °C/min. Solubility studies were analogous to the TGA isothermal experiments with 10 mg of the starch heated at 160 °C for 6 h followed by addition of 1 mL of anhydrous DMSO. Dissolution was attempted by alternating heat (<80 °C) and agitation with a vortex mixer.

3. Results and discussion

3.1. Substitution of LiN_3

Cimecioglu et al. (1994) have found that lithium salts such as LiBr and LiCl can aid in the dissolving of amylose in DMF. They have also found the same enhancement with lithium azide (Cimecioglu et al., 1997). Because LiN_3 was available only in an aqueous solution, initial attempts of the azidation reaction used only anhydrous NaN_3. Following Cimecioglu and co-workers' procedure (Cimecioglu et al., 1997) with BioChemika amylose using one equivalent of reagents, only a 5% conversion of the amylose to its azide derivative was observed. As a result, anhydrous LiBr or LiCl was added to enhance dissolution of the amylose. The added LiBr salt with NaN_3 appeared to aid in dissolving BioChemika amylose, and 6-azido-6-deoxyamylose was observed at 40% conversion, similar to literature data (Cimecioglu et al., 1997). However, anhydrous lithium salts are very hygroscopic and they visibly absorbed water despite being stored in a dessicator. Subsequent use of the salt resulted in lower and lower conversions of starch to the desired product. This is not too surprising because researchers have found that moisture can detrimentally affect the conversion of alcohols to nitriles using similar reagents (PPh_3, CCl_4, and NaCN) (Brett, Downie, & Lee, 1967).

To avoid the use of hygroscopic lithium salts, the reaction was attempted utilizing only sodium azide with the dissolution temperature raised to 100 °C from 80 °C for 1 h. With one equivalent of reagents to BioChemika amylose at the increased temperature, NaN_3 worked similar to LiN_3, producing 40% conversion. When increased to two equivalents of reagents to amylose, full conversion (as observed by ^{13}C NMR) of the amylose to the corresponding 6-azido-6-deoxyamylose was obtained with 75% recovery. The amylose product was characterized by FT-IR and ^{13}C NMR, which matched that of published data (Cimecioglu et al., 1997, 1994). Fig. 1 shows the ^{13}C NMR of amylose (top) and the reaction product (bottom). Thus, NaN_3 has been shown to be a good substitute for LiN_3 in the azidation reaction.

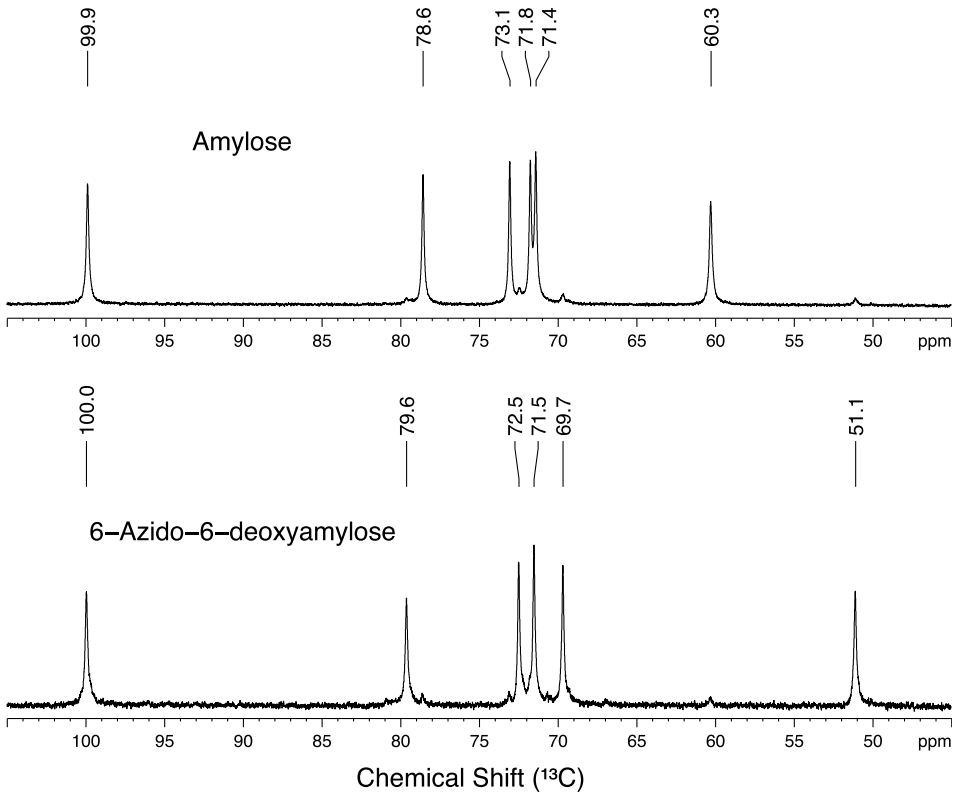


Fig. 1. ^{13}C NMR spectra of amylose (top) and 6-azido-6-deoxyamylose (bottom).

3.2. Scope of the reaction

Knowledge of the scope of this reaction is important because the derivatized starch will be utilized in material applications. Furthermore, the use of industrial starches instead of only amylose would be more practical and cost effective. Thus, the reaction was extended to include amylopectin, high amylose corn starch (HACS), potato starch, wheat starch, and waxy corn starch.

Amylopectin reacted to produce the desired 6-azido-6-deoxyamylopectin (100% conversion) under typical reaction conditions (two equivalents of reagents) with recoveries similar to amylose (75%). FT-IR spectra of both the starting amylopectin (dotted line) and reacted amylopectin (solid line) are shown in Fig. 2. The reacted amylopectin has a peak at 2100 cm^{-1}, which is identical to the azide peak for reacted amylose. Furthermore, the decreases at ~3300 and ~1100 cm^{-1} signify a reduction in alcohol groups on the starting material. The selective C-6 conversion was confirmed by ^{13}C NMR spectroscopy, which is shown in Fig. 3. The top half shows the ^{13}C NMR spectra of the amylopectin starting material, while the bottom half is the reacted product. The peak positions for both starting material and product are similar to those of amylose (Cimecioglu et al., 1997, 1994; Falk, Micura, Stanek, & Wutka, 1996). Specifically, the C-6 resonance of amylopectin shifts from 60 ppm in the starting material to 51 ppm in the azide product. The smaller side peaks in the amylopectin spectra is due to the branching at the C-6 position (Falk et al., 1996). As with amylose, no evidence of substitution at C-2 or C-3 was observed.

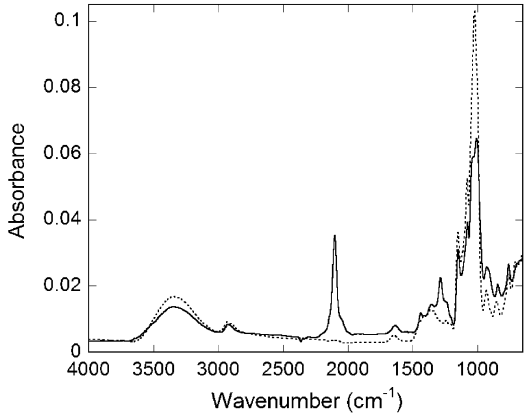


Fig. 2. FT-IR spectra of amylopectin (dotted line) and 6-azido-6-deoxyamylopectin (solid line).

Extension of this reaction to industrial starches is of more practical use since amylose and amylopectin are both relatively expensive. To this end, derivatization of native potato starch, native wheat starch, and native HACS were attempted under the typical reaction conditions with two equivalents of reagents. Interestingly, none of these starches reacted to provide the desired product. The reason is likely due to solubility because the native starches did not appear to dissolve in the reaction. However, when pregelatinized potato, wheat, and

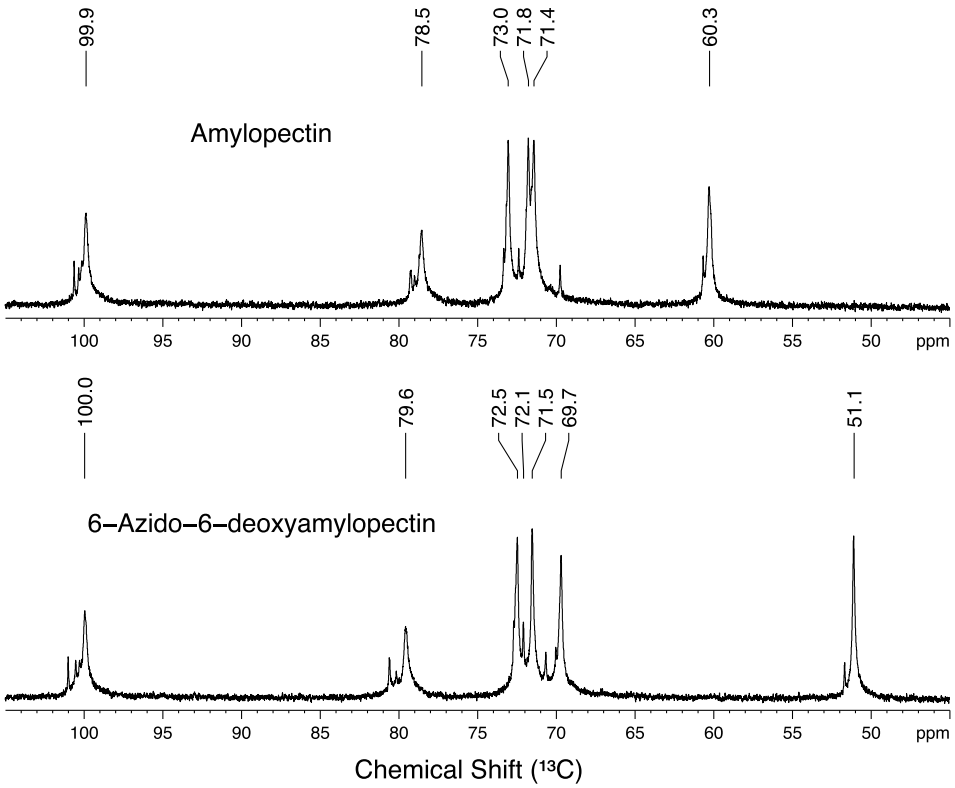


Fig. 3. ^{13}C NMR spectra of amylopectin (top) and 6-azido-6-deoxyamylopectin (bottom).

HACS were reacted under the typical reaction conditions with two equivalents of reagents, conversions of 93%, 100%, and 88%, respectively, were obtained. These results suggest that industrial starches will dissolve in DMF at elevated temperatures when their granular structure has been disrupted.

3.3. Reaction rate

Cimecioglu et al. (1997) showed that the azidation of amylose is complete when allowed to proceed overnight. This azidation reaction would likely be more practical if it can proceed at a faster rate. Before attempting to increase the rate of reaction, the time for the reaction to reach completion was determined. Thus, the extent of amylose azidation was monitored by taking aliquots as the reaction proceeded. Fig. 4 shows the reaction progress as monitored by ^{13}C NMR comparing the C-6 carbon peaks at 51 and 60 ppm. The reaction setup was the same as that of a typical reaction with two equivalents of reagents at ten times the scale. The result showed that the reaction was complete after 1 h. The lag that was seen at the first 20 min was due to warming of the reaction from 0 °C to room temperature. It is interesting to note that the reaction did not proceed at 0 °C as evidenced by the first data point. No evidence of intermediates or side products, such as 6-bromo-6-deoxyamylose, was found in the reaction. With amylopectin, the reaction was also observed to be complete within 1 h.

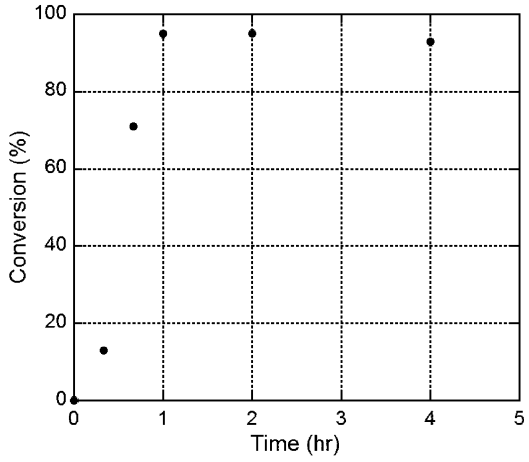


Fig. 4. Reaction progress of amylose monitored by ^{13}C NMR. Conversion was determined by comparing the C-6 carbon peaks of the starting amylose (60 ppm) and the azide product (51 ppm).

3.4. Substituting for DMF

A goal of this project involves the extension of this reaction to other biopolymers. One important factor to consider in this reaction is the solvent. Because biopolymers have differing solubility in different solvents, it was desirable to know if the azidation reaction could be carried out in solvents other than DMF. For example, cellulose and starches have been derivatized in solutions of *N*,*N*-dimethylacetamide (DMAc) with LiCl (McCormick, 1981). If this

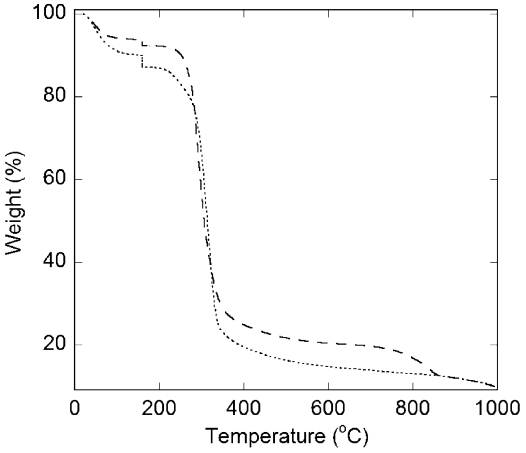


Fig. 5. Thermograms of unmodified amylose (dashed line) and 5% azide-substituted amylose (dotted line). Samples were heated at 20 °C/min from room temperature and held at 160 °C for 6 h.

azidation reaction could also utilize DMAc as a solvent instead of only DMF, then the reaction can be extended to cellulose or other biopolymers that contain a primary hydroxyl group. Under the typical reaction conditions using two equivalents of reagents with DMAc, 81% and 100% conversion of amylose and amylopectin, respectively, were obtained.

3.5. Thermal properties

Preliminary TGA and solubility studies were conducted to determine the effect of azidation on the properties of starch. TGA experiments showed that degradation of the azide group started at approximately 160 °C. Unmodified amylose (with the same thermal history as the modified amylose) and 5% azide-substituted amylose were heated as described in the experimental section, and the results are shown in Fig. 5. The dashed line is from unmodified amylose, whereas the dotted line is from 5% substituted amylose. The substituted amylose lost more weight when held isothermally at 160 °C suggesting N_2 emission from azide decomposition. At higher temperatures, the peak value of the derivative of the weight change was 290 °C for unmodified amylose and 315 °C for substituted amylose. This indicated that the substituted amylose has increased thermo-stability compared to the unmodified amylose.

Solubility studies of 5% substituted amylose and unmodified amylose were performed as stated in the experimental section. The substituted azide did not appear to dissolve, whereas the unmodified amylose easily dissolved. While these results are qualitative, they suggest that azide decomposition does result in cross-linked products. Thus, azide substitution onto the starch backbone can be thermally activated to produce a cross-linked carbohydrate with modified properties. Further experimentation on the material properties of derivatized starches will be reported in due course.

4. Conclusions

The selective azidation of starch was extended to include sodium azide as the source of the azide group. Most importantly, the starches that can be derivatized have been expanded to include amylopectin and industrial starches. Furthermore, DMAc was found to be a possible solvent alternative to DMF in these reactions, paving the way to the use of other biopolymers. Under our conditions, the reactions were determined to be complete within 1 h. Currently, the material properties that these derivatives impart when cross-linked are being studied. Plans to extend this reaction to other underutilized biopolymers are underway.

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