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Sub-T_g thermal properties of amorphous waxy starch and its derivatives

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The sub-T_g thermal properties of aged amorphous waxy starch, β-limit dextrin, and amylopectin triacetate at low moisture contents were studied by differential scanning calorimetry (DSC). When T_g was above 50°C (~5–14% moisture), a 50°C endothermic peak was observed in waxy starch and β-limit dextrin samples. The ΔH of the 50°C peak was proportional to the moisture content. At a similar moisture content the ΔH of β-limit dextrin was greater than that of waxy starch. No 50°C peak was observed in the amylopectin triacetate samples at the moisture contents studied (~0·5–5% moisture). All amylopectin triacetate samples showed an endothermic overshoot associated with the glass transition. It is suggested that water–hydroxyl group interactions may be required for the formation of the sub-T_g peak. The 50°C endotherm is a separate event different from the aging peak associated with the glass transition seen in many polymers.

INTRODUCTION

An enthalpic thermal event near the glass transition temperature (T_g) is common in synthetic polymers (Sperling, 1992). In polymers aged at a sub-T_g temperature the thermal event is usually observed as an endothermic differential scanning calorimetry (DSC) peak concomitant with or immediately prior to the glass transition. The endothermic peak may reflect an enthalpy relaxation of a glass upon sub-T_g aging. Hodge & Berens (1981) summarized several general features of the sub-T_g endotherm due to an enthalpy relaxation: (1) It increases in magnitude and shifts to higher temperature (T_{max}) with increasing annealing time (t_e) and annealing temperature (T_e). (2) At long t_e and/or high T_e it merges with the glass transition and becomes the well-known endothermic overshoot. (3) The time t_e required to produce a given peak height decreases with increasing T_e. (4) T_{max} is proportional to T_e at constant t_e and approximately proportional to $\log(t_e)$ at constant T_e. Alternatively, a sub-T_g endothermic peak may result from a first order melting transition. This is especially possible for a system that has various types of amorphous segments. The system might contain incompatible polymers with different T_gs, or it might contain the same general type of polymer in a heterogeneous sample. In the latter case, multiple amorphous domains of different size, with different plasticizer contents and T_g values, could coexist (Slade & Levine, 1994). Some of the minor T_gs might be below the storage temperature, therefore enabling the crystallization of some amorphous segments in the system during storage.

The glass transition behavior of food polymers was not extensively studied until the middle of the past decade. It is not surprising that reports about a sub-T_g thermal event in food materials have only just surfaced in the literature. Kalichevsky *et al.* (1992) first reported the observation of a sub-T_g DSC endothermic peak at 50°C for aged amorphous amylopectin with about 10–15% moisture. A similar endothermic peak was also reported by Shogren (1992) for native corn starch and aged gelatinized corn starch with about 10–20% moisture. Based on the observation that CP/MAS C-13 NMR spectra of corn starch before and after aging were nearly identical, Shogren (1992) concluded that the endothermic peak seen in starch was due to enthalpy relaxation rather than melting of a helix or other highly organized structure. Appelqvist *et al.* (1993) observed the endothermic peak in a broad range of polysaccharides at low moisture content regardless of whether or not the system showed evidence of a glass transition. These authors suggested that the endothermic event reflected the disruption of energetic association between water molecules and hydrophilic

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groups on the polysaccharide under conditions of restricted mobility. Using DMTA analysis, these authors also observed a peak in tan δ at 50–60°C. Based on the fact that the tan δ peak was virtually frequency independent, they inferred that the event was more characteristic of a structural melting than a relaxation process. Some features of the sub-T_g endothermic peak reported for starch include: (1) it only appears on storage in the presence of limited moisture contents when the T_g is above the peak maximum temperature (Kalichevsky *et al.*, 1992; Shogren, 1992; Appelqvist *et al.*, 1993), (2) peak maximum temperature is relatively constant irrespective of changing T_g (Appelqvist *et al.*, 1993), and (3) ΔH increases with increasing moisture content (Shogren, 1992; Appelqvist *et al.*, 1993). A sub-T_g endothermic peak has also been found in aged wheat gluten (Lawton & Wu, 1993). However, unlike with starch, the peak observed in aged gluten was always associated with the T_g, more characteristic of the endothermic overshoot described by Sperling (1992).

With very limited evidence available in the literature, it is still a point of contention whether the endothermic event below the T_g of polysaccharides reflects structural melting or enthalpy relaxation. The purpose of this paper is to further explore the nature of the peak by examining the thermal properties of waxy starch and two derivatives, namely β-limit dextrin and amylopectin triacetate. Because of its reduced tendency to form double helices, β-limit dextrin was examined to determine whether the peak is related to double helices. If double helices are related to the sub-T_g peak, one should observe little or no sub-T_g peak in an aged β-limit dextrin sample. Amylopectin triacetate, having all free hydroxyl groups substituted with acetyl groups, was chosen to study the role of the starch hydroxyl groups.

EXPERIMENTAL

Amorphous waxy starch

A starch paste was prepared from a 5% slurry (w/w) of waxy starch (Amioca, American Maize-Products Co., Hammond, IN) by heating in a Brabender VISCO/amylo/GRAPH from 5 to 95°C at 1·5°C/min, followed by holding at 95°C for 20 min and then cooling at 1·5°C/min to room temperature. A portion of the paste was spread into a 5 mm layer in a glass tray and dried in an oven at 130°C for about 4 h, when it became glassy. The dried starch sample was ground into coarse powder and stored in a desiccator for later use.

β-Limit dextrin

β-Limit dextrin of waxy starch was prepared according to the method of Whelan (1964) with only slight modification. A starch slurry was prepared by mixing 3 g (dry weight) of waxy starch with 500 ml deionized water (containing 0·02% sodium azide as preservative) and 10 ml of 3 N sodium hydroxide in a 1 liter flask. The starch slurry was then gelatinized in a boiling water bath 30 min with stirring. After cooling to room temperature, the pH of the starch suspension was adjusted to 4·8 with 3 N sulfuric acid solution followed by the addition of 60 ml of 0·2 M (pH 4·8) sodium acetate buffer. The suspension was then inoculated with 5000 units of β-amylase (EC 3.2.1.2; product number A7005, Sigma Chemical Co., St Louis, MO) and incubated in a 37°C water bath shaker. After 24 h the β-amylase was inactivated by heating in a boiling water bath for 10 min. The digest was concentrated to 300 ml by boiling, and then dialyzed using membrane tubing (MW cutoff: 6000–8000; Spectrum Medical Industries, Los Angeles, CA) in 50 liters of deionized water for 48 h, with one water change at 24 h. After the dialysis, the digestion procedure was repeated to ensure that complete hydrolysis was obtained. After the second dialysis, the dialysate was concentrated to about 150 ml by boiling. The β-limit dextrin was then precipitated by adding the dialysate into 450 ml of vigorously stirred methanol and collected by centrifuging at 900 *g* for 10 min. The β-limit dextrin was dried under reduced pressure (80 mm Hg, absolute) at 95°C for 12 h and ground to coarse powder for later use.

Amylopectin triacetate

Acetylation of the aforementioned waxy starch was based on the method described by Hodge (1964). Four grams of thoroughly dried (100°C for 12 h at 80 mm Hg absolute pressure) waxy starch was heated in 50 ml of refluxing pyridine at 115°C for 1 h. The suspension of activated starch granules in pyridine was cooled at room temperature for 5 min and then stirred while 13·2 ml of acetic anhydride was added dropwise. The suspension was heated in a 100°C oil bath for 2 h. The final product was a transparent and viscous paste. After cooling to room temperature, the paste was transferred into 400 ml of vigorously stirred methanol. The white precipitate was isolated by centrifugation at 900 *g* for 10 min and washed twice with 100 ml methanol and five times with 75 ml absolute ethanol. The starch triacetate was air dried at room temperature for 4 h, followed by drying at 95°C at 80 mm Hg for 12 h.

Differential scanning calorimetry (DSC)

Approximately 10 mg of each of the samples was dried in an individual preweighed stainless steel DSC pan (Perkin-Elmer, Norwalk, CT) at 110°C in a vacuum oven (80 mm Hg) for 24 h, followed by storage in a desiccator containing phosphorous pentoxide (P_2O_5) at room temperature for another 24 h. After the dry starch weight was determined, the samples were conditioned at

room temperature in desiccators with different RHs for 4 days. The RHs, controlled by various saturated salt solutions, were 23, 35, 43, 65, 75 and 94%. After conditioning, the samples were sealed in the DSC pans and weighed to determine the moisture contents. The samples were further conditioned in the sealed DSC pans at room temperature for 1 day. The samples were then examined using a DSC (Perkin-Elmer DSC 7) equipped with a thermal analysis data station. Samples were heated at a rate of 10°C/min in the DSC with a sealed empty pan as the reference. The thermograms were normalized based on dry sample weight. Glass transition temperature (T_g), peak temperature (T_{max}) and enthalpy (ΔH) were calculated with the thermal analysis software program.

RESULTS

Figures 1 and 2 show the representative DSC thermograms of aged waxy starch and its β-limit dextrin, respectively, at six moisture contents. The two sets of samples are similar in that a 50°C endothermic peak

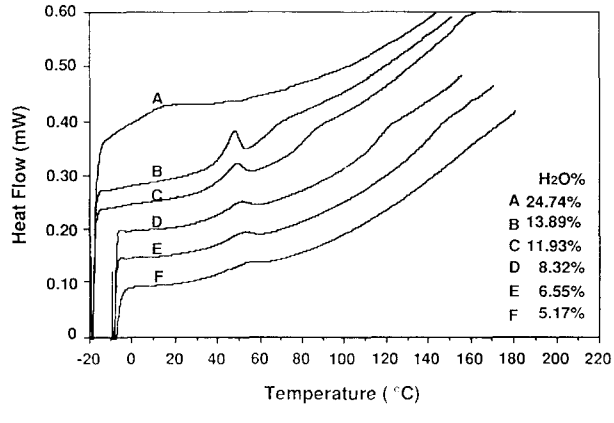

Fig. 1. DSC thermograms of amorphous waxy starch aged at various moisture contents. Each scan has been normalized based on dry sample weight.

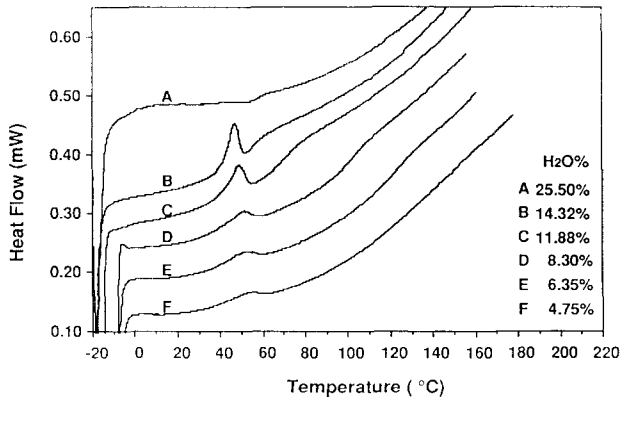

Fig. 2. DSC thermograms of β-limit dextrin of waxy starch aged at various moisture contents. Each scan has been normalized based on dry sample weight.

was observed in samples with a T_g above 50°C. The peak was not observed upon immediate reheating in the DSC (Fig. 3). These results are consistent with the finding of Appelqvist *et al.* (1993) for amylopectin aged at room temperature. For those samples that show a sub-T_g peak, the T_g and T_{max} (from two analyses) are plotted versus corresponding moisture contents in Fig. 4. The T_g is highly moisture dependent due to the plasticizing effect of water. The T_g of the β-limit dextrin is lower than that of waxy starch at a given moisture content, perhaps because of the reduction in average chain length and molecular weight. On the other hand, the T_{max} of the sub-T_g peak is relatively insensitive to moisture content and, thus, to the change in T_g. Although moisture has little effect on the T_{max}, it is directly related to the enthalpy of the sub-T_g peak. A plot of ΔH (based on dry weight) versus moisture content (Fig. 5) shows that ΔH increases linearly with increasing moisture content. Figure 5 also shows that ΔH of β-limit dextrin at a given moisture content is greater than that of waxy starch.

Complete acetylation of the hydroxyl groups on starch molecules renders the starch insoluble in water (Jarowenko, 1986). As a result of decreased water affinity, the post-conditioning moisture contents of the starch triace-

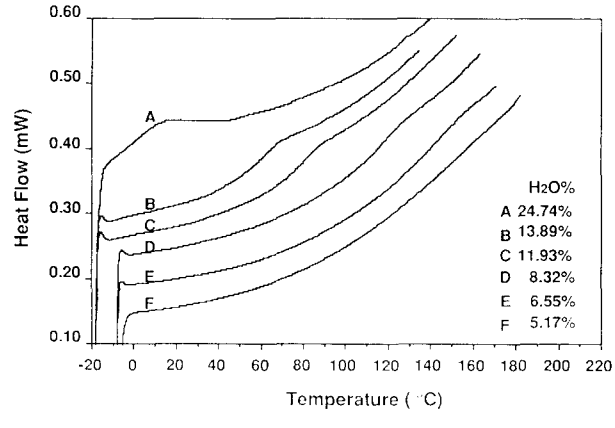

Fig. 3. Immediate DSC rescans of samples shown in Fig. 1.

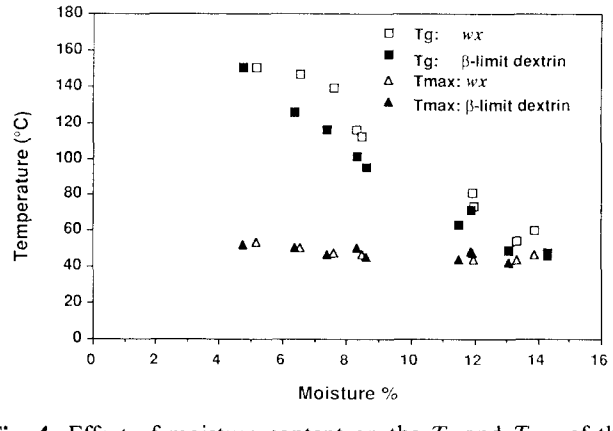

Fig. 4. Effect of moisture content on the T_g and T_{max} of the sub-T_g peak of aged waxy starch and β-limit dextrin.

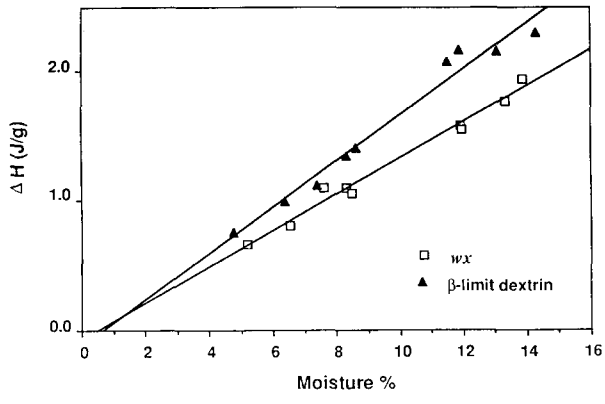

Fig. 5. ΔH of the sub-T_g peak of aged waxy and β-limit dextrin as a function of moisture content.

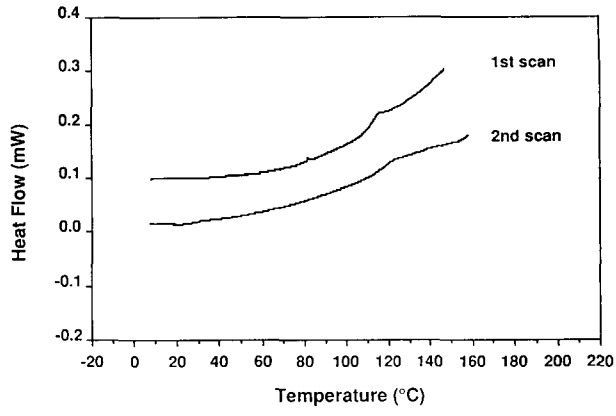

Fig. 7. Initial DSC thermogram and immediate rescan of aged amylopectin triacetate with 41% moisture.

tate (~0·5–5%) were much lower than the native amorphous waxy starch (~5–25%). DSC thermograms of the starch triacetate at six moisture levels are shown in Fig. 6. All six thermograms show an overshoot peak that coincides with the glass transition, typical of the sub-T_g peak observed in aged synthetic polymers (Sperling, 1992). Unlike the sub-T_g peak found in the waxy and β-limit dextrin samples, it appears that the magnitude of the peak is not related to water content. This peak did not appear upon immediate rescanning (data not shown). No sub-T_g peak was observed at 50°C, even for the sample with 4·9% moisture (Fig. 6A), whereas a peak was observed for waxy starch and β-limit dextrin at a similar moisture content (Figs 1F and 2F). Although no 50°C peak was observed in the triacetate samples with moisture content up to 4·9%, it is conceivable that the peak might appear at a higher moisture content. To examine this possibility, three triacetate samples were mixed with water and then conditioned in sealed DSC pans at room temperature for 4 days before being examined with DSC. Moisture contents of the samples, which were determined after DSC analysis, ranged from about 39 to 41%. Figure 7 shows one representative DSC thermogram and its immediate rescan. Even at such a high moisture content, there was no 50°C

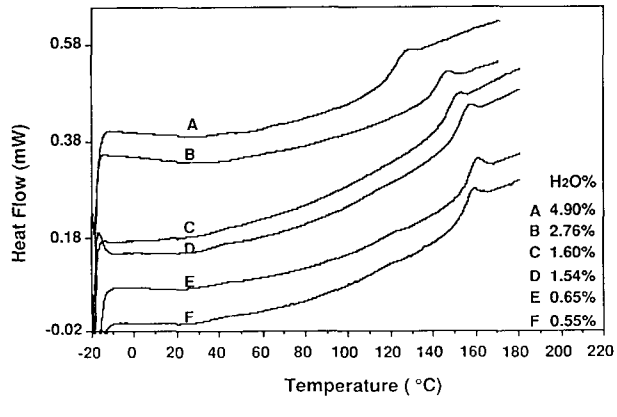

Fig. 6. DSC thermograms of amylopectin triacetate aged at various moisture contents. Each scan has been normalized based on dry sample weight.

peak observed and the T_g of the sample (~112°C) was close to that at 4·5% moisture (~118°C). This result indicates that water is not an effective plasticizer for starch triacetate and suggests that much of the added water may be in a separate phase.

DISCUSSION

Effect of β-amylolysis

Since the formation of double helices in β-limit dextrin is greatly inhibited, the DSC results (Figs 2 and 5) for the β-limit dextrin rule out the possibility that double helices are responsible for the formation of the sub-T_g peak. The finding that the ΔH (based on dried starch weight) of β-limit dextrin is higher than waxy starch (Fig. 5) suggests that the reduced chain length of amylopectin might actually enhance the formation of the sub-T_g peak. Hodge & Berens (1981) pointed out that the magnitude of a sub-T_g relaxation peak increases with increasing sub-T_g aging temperature (T_e) mainly due to a greater relaxation rate at a higher aging temperature. Since the T_g of the β-limit dextrin was lower than that of the waxy starch at the same moisture level, the difference between T_g and T_e was therefore greater for the β-limit dextrin than the waxy starch. As a consequence, the relaxation rate would be higher for the β-limit dextrin at T_e, resulting in the higher ΔH compared to the waxy starch. The results from the β-limit dextrin samples seem to support the idea that the peak is due to enthalpy relaxation and negates the possibility that double helices are related. Shogren (1992) came to the same conclusion based on the fact that no structural change in the starch backbone was observed after heating the aged starch sample.

Effect of acetylation

The disappearance of the 50°C endothermic peak after complete acetylation of waxy starch suggests the

hydroxyl groups play a role in the formation of the peak. This result is consistent with the assessment of Appelqvist *et al.* (1993) that the sub-T_g peak reflects the disruption of energetic interaction between water and hydrophilic groups on various biopolymers. An interesting observation for the triacetate samples was that all six samples exhibited an endothermic overshoot at the glass transition (Fig. 6) that was not observed in the native waxy starch. Unlike the 50°C peak, the magnitude of this endothermic overshoot was not affected by water content and was more typical of the sub-T_g relaxation peak seen in most synthetic polymers. Since the T_gs of the triacetate samples were all above 110°C, the drying step (110°C, 24 h) might have effected the enthalpy relaxation through sub-T_g aging. The motion of the acetyl groups is probably the underlying cause of this observation. This phenomenon is consistent with the theory of Smith & Boyd (1992), who suggested that reorientation of flexible side groups might be an important cause of a sub-T_g relaxation.

Role of water

The fact that the ΔH increases with increasing water content for amylopectin and its β-limit dextrin (Fig. 5) underscores the importance of water in the 50°C sub-T_g peak. There are two possible mechanisms by which the water molecules can influence the magnitude of the sub-T_g peak. First, the involvement of water might be through a kinetic mechanism, in that the water molecules increase the enthalpy relaxation rate of the glass by providing greater local mobility (i.e. decreasing (T_g–T_e)). Second, the water molecules might be directly involved through the formation of a specific structure that accounts for the sub-T_g peak. Gidley *et al.* (1993) have suggested that the 50–70°C endotherm observed in many polysaccharides reflects the disruption of a hydrogen-bonded network involving water and polysaccharides.

An intriguing feature of the sub-T_g peak observed in waxy starch and β-limit dextrin is that the T_{max} remains relatively constant in spite of the changing T_g, which was observed over a wide range of about 100°C. On the other hand, for amylopectin triacetate the location of the endothermic peak changes with changing T_g (Fig. 6). The invariability of the peak location in waxy starch and β-limit dextrin could be explained by the presence of a specific association involving water in these samples.

A further experiment was conducted to probe the central role of water in the 50°C thermal event: dry glassy waxy starch samples were prepared and conditioned as described above at 43, 65, and 75% RH. After the 4-day conditioning period, a sample at each RH was sealed in a DSC pan and the moisture content was determined. As shown previously, glassy starch samples conditioned at these conditions should show a 50°C endotherm when examined by DSC. Another sample at each RH was removed from the desiccators and put into a desiccator containing P_2O_5. After 7 days in the desiccator at room temperature, samples were sealed and weighed. This treatment reduced the moisture contents of the samples from about 10, 13 and 15% for RH's of 43, 65, and 75%, respectively, to about 2%. Samples were then examined by DSC. Representative DSC thermograms are shown in Fig. 8. It is clear that drying the RH-conditioned samples above P_2O_5 at room temperature effectively eliminated the 50°C sub-T_g peak. The theory of enthalpy relaxation suggests that a sub-T_g endotherm reflects the recovery of entropy that was lost during slow cooling or sub-T_g aging (Noel *et al.*, 1993). It is difficult to imagine how loss of moisture would lead to recovery of lost entropy, as would be necessary to account for the loss of an endotherm due to enthalpy relaxation. This observation strongly supports the hypothesis that disruption of water–starch interactions, rather than enthalpy relaxation, is the underlying cause of the 50°C endothermic event. These putative interactions, possibly involving water and starch hydroxyl groups, might be disrupted in three ways: (1) heating the sample above the dissociation temperature, in this case 50°C, (2) removing water from the sample by reducing the water vapor pressure above the sample (e.g. using P_2O_5), and (3) drastically increasing the backbone mobility of the glassy polymer by increasing the moisture content so T_g is below 50°C. We propose that this 50°C endotherm is a separate event different from the aging peak associated with glass transition seen in many polymers.

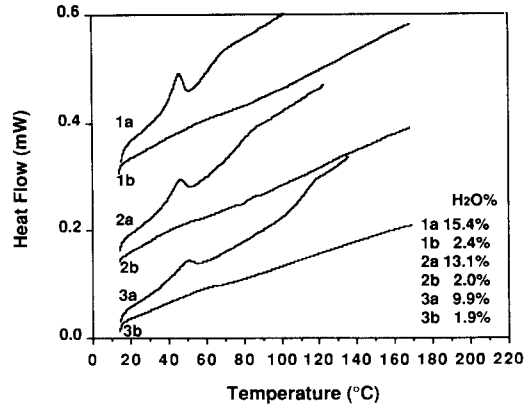

Fig. 8. DSC thermograms of amorphous waxy starch conditioned at various conditions. 1a: 4 days at 75% RH + 7 days in sealed DSC pan. 1b: 4 days at 75% RH + 7 days above P_2O_5. 2a: 4 days at 65% RH + 7 days in sealed DSC pan. 2b: 4 days at 65% RH + 7 days above P_2O_5. 3a: 4 days at 43% RH + 7 days in sealed DSC pan. 3b: 4 days at 43% RH + 7 days above P_2O_5.

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