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Influence of Amylose Content on Formation and Characteristics of Enzyme-resistant Starch

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Abstract

Influence of amylose content on formation and characteristics of enzyme-resistant starch (RS) was investigated by scanning electron microscopy, X-ray diffractometry and differential scanning calorimetry. RS yield increased up to 36.1% as the amylose content of corn starch increased. Starch granules of Amylomaize V and VII were more rounded and smaller than those of regular corn: some were elongated and had appendages. After autoclaving-cooling cycles, the granular structure disappeared and a continuous spongy-like porous network was visible in regular corn starch; the granular structure was still evident in parts in Amylomaize V and VII starches. In all isolated RS residues, the porous structures were no longer visible and more compact formations predominated. While regular corn starch showed an A-type X-ray profile, Amylomaize V and VII starches exhibited a combination of B- and V-types. Regular corn starch lost most of its crystallinity during autoclaving, but the crystallinity was still left in Amylomaize starches as diffuse or poor B-types. All RS residues showed the presence of poor B-type regardless of amylose contents. Transition temperatures and enthalpy of native starches were a little higher in Amylomaize V and VII starches than those of regular corn starch. Regardless of amylose contents, all RS residues exhibited an endothermic transition over a similar temperature range (135~169°C), with a mean peak temperature of ~154°C, which is generally found for retrograded amylose crystallites. Higher transition temperature, enthalpy, and RS yield of Amylomaize V and VII starches were related to granular stability shown by the microscopic and crystallographic studies.

Key words: enzyme-resistant starch, yield, B-type, retrograded amylose crystallite

INTRODUCTION

Starch, a major component in cereal grains, roots, tubers, and some legumes, is an important energy source in the diet of man. It consists mainly of two D-glucosyl polymers: amylose and amylopectin. Amylose is primarily a linear molecule with α-(1,4) linkages. Some amylose molecules, particularly those with large molecular weight, may have up to 10 or more α-(1,6) branches. The degree of polymerization (DP) is in the range of 500~6,000 glucose residues (1). Amylopectin is a highly branched molecule, with α-(1,4) linked backbones and α-(1,6) branch points (4~5% of the glycosidic bonds). Three types of chains can be distinguished: short 'A' chains with DP of 14 to 18 glucose residues, organized in a cluster structure, inner long 'B' chains (DP 45~55), and a single chain of DP higher than 60 bearing a reducing end-group. The DP of amylopectin is ~10^5 glucose residues (2). *In vivo*, starch polymers are hydrolysed by excess levels of pancreatic α-amylase (EC 3·2·1·1). A portion of starch in starch-based foodstuffs, however, escapes digestion in the small intestine of humans according to degree of gelatinization, granule size, amylose/amylopectin ratio, starch-protein interactions, amylose-lipid complexes, and percentage of retrograded starch (3). This fraction is called enzyme-resistant starch (RS) and has properties similar to those of dietary fiber. Eerlingen and Delcour (2) classified RS into four different types. Type I RS represents physically inaccessible starches in partly milled grains and seeds and in legumes, which are locked in the plant cell. Type II RS is a native granular starch found in raw potatoes and bananas. Type III RS is an indigestible starch fraction which is formed after certain heat-moisture treatments of the starch and may be present in the products such as cooled or cooked starch foods (retrograded amylopectin and/or retrograded amylose). Finally, Type IV RS may be formed by chemically or thermally modifying the starch. Formation of glycosidic bonds other than α-(1,4) or α-(1,6) by heat treatment (e.g. caramelization and Maillard reactions) reduces the availability for amylolytic enzymes. Also, cross-linking or the presence of some substituents (e.g. hydroxy propyl) may reduce the digestibility of the starch.

Interest in RS results from its inclusion in insoluble dietary fiber when applying an AOAC enzymatic-gravimetric method (4). Physical and chemical characterizations have been carried out to explain formation of RS (5-12). Accordingly, these investigations indicate that mainly retrograded and recrystallized amylose is involved in formation of Type III RS. High levels of RS can be produced during autoclaving and cooling of high-amylose starch (3,13).

A high-fiber, low-fat, and low-calorie product is an important objective in today's food development. From a physio-

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logical standpoint, however, RS is still in the evaluation stage as a natural dietary fiber. The objective of this study was to investigate the influence of amylose content on formation and characterization of enzyme-resistant starch by microscopic, crystallographic, and thermodynamic methods.

MATERIALS AND METHODS

Commercially available starches were used to produce RS: regular corn starch from Samseung Co., Korea and high amylose corn starches (Amylomaize V and VII) from American Maize Products Co., Hammond, IN. For the isolation of RS, heat stable α-amylase (A-3306), protease (P-5380), and amyloglucosidase (A-9913) were purchased from Sigma Chemical Co., St. Louis, MO.

Chemical analyses

Proximate composition of starch samples was determined according to AACC method (14). Quantification of amylose was done from the blue value at 680 nm according to Gilbert and Spragg (15) modified by Suzuki et al. (16). All analyses were done at least in triplicate; average results were given on a dry matter basis.

Formation and isolation of RS

RS was formed in an aqueous system (starch : water=1 : 3.5), after four autoclaving (121°C, 1 hr) and cooling (4°C, 22 hrs) cycles, from starch samples, according to the method adapted from Sievert and Pomeranz (3). A product, containing RS was freeze-dried and ground in a mortar to pass a sieve with 500 μm diameter openings. The technique of RS isolation was based on the AOAC method for the dietary fiber determination (4) as described by Eerlingen et al. (17). The isolated RS residues were freeze-dried and the yields were calculated as a percentage of the starch used for enzyme hydrolysis. The dried material was ground in a mortar for further use to pass a sieve with 500 μm-diameter openings.

Scanning electron microscopy (SEM)

Morphological changes of starch samples during the formation of RS were observed with a Hitachi X-650 (Japan) scanning electron microscope operated at 15 kV. SEM micrographs were analyzed to measure the size of starch granules using the Image-Pro Plus program (Media Cybernetics, USA).

X-ray diffractometry

X-ray powder diffraction analysis was performed with a MXP18 diffractometer (MAC Science, Japan). Operating conditions were as follows: target: Cu-Kα; scanning speed: 0.5°/min; voltage: 40 kV; current: 100 mA. Diffractograms of the samples were obtained at 2θ ranging from 5° to 40°.

Differential scanning calorimetry (DSC)

Measurements were performed at least in triplicate with a TA-2000 differential scanning calorimeter (TA Instruments, USA). About 3 mg starch sample was accurately weighed into a hermetic aluminum pan and two times the sample weight of water was added, and then it was allowed to equilibrate for an hour at ambient temperature. An empty pan served as the reference. The DSC was heated from 30 to 200°C at a heating rate of 10°C/min. For each endotherm, the onset (To), peak (Tp), completion (Tc) transition temperatures, and enthalpy (ΔH) were determined.

RESULTS AND DISCUSSION

Effect of amylose content on Type III RS yield

The proximate chemical composition, amylose content, and Type III RS yield of starches are presented in Table 1. Crude protein content increased in proportion to amylose content.

Amylose content and the yield of Type III RS were positively correlated (r=0.994) as in other recent reports (3,6,18). The highest yield of RS (36.1%) was obtained with Amylomaize VII, the starch with the highest amylose content (67.0%). This is evident when one considers the formation of Type III RS to be a retrogradation or crystallization process of amylose. Yields in excess of 20% RS were obtained from autoclaved high amylose starches. They may even be raised to the level of 40% by increasing the number of autoclaving-cooling cycles (3,18).

The yields of RS in starches were also affected by water content and autoclaving temperature. A maximum in RS yield was found when starch : water ratio was 1 : 3.5 (w/w) (3). A minimum of water, however, is necessary for plasticisation of the environment and for incorporation into the crystal structure. By X-ray diffractometry studies, B-type amylose crystal is known to contain 36 water molecules and 12 anhydrous glucose units (19). Therefore, it contains about 27% water. The influence of the autoclaving temperature varies with starch type. An increase in RS yield was observed in wheat starch when the temperature was increased from 100°C to 134°C while no significant differences were observed from amylomaize starch (7). Autoclaving at 148°C leads to a decrease in RS yield (3,6). Indeed, at higher temperatures, the crystal growth rate is favored (2, 12). Autoclaving at 148°C, however, results in crystal melting.

Morphological characteristics by SEM

Starch granules of regular corn were polygonal and/or spherical, but those of Amylomaize V and VII were generally more rounded and smaller; some were elongated and had append-

Table 1. Type III RS yield and chemical composition of starches with different amylose content (%)

Type of starch	Moisture	Crude protein	Crude lipid	Crude ash	Carbohydrate	Amylose	RS yield
Regular corn	8.78	0.28	0.18	0.17	90.59	25.9	14.5
Amylomaize V	11.45	0.45	0.08	0.10	87.92	46.6	27.8
Amylomaize VII	10.89	0.48	0.12	0.15	88.36	67.0	36.1

ages (Fig. 1). The sizes of granules became smaller, from an average diameter 13.9 to 9.1 μm as amylose content of starch increased (Table 2). After autoclaving-cooling cycles, the granular structure of corn starch disappeared and a continuous spongy-like porous network with an irregularly shaped swollen particles was visible. In Amylomaize V and VII starches, this porous structure was predominant as in regular corn starch, but granular structure were still evident in parts even after four autoclaving-cooling cycles (Fig. 1). In all RS residues isolated after enzyme digestion, the porous structures were no longer visible since they were most likely removed by the enzymes and very compact and dense formations predominated regardless of amylose content (Fig. 1). Similar morphological changes were also observed in the oven-dried RS residues by Sievert and Pomeranz (3). Recently, Mun et al. (18) reported that RS crystals were isolated. In this study, we also found the same shaped crystals on the surface of isolated RS residues shown in scanning electron micrographs. By X-ray diffractometry, however, they were confirmed to be glucose monohydrate

Table 2. Sizes and shapes of starches

Type of starch	Size range (μm)	Size average (μm)	Shapes
Regular corn	8.0~21.2	13.9	polygonal, spherical
Amylomaize V	6.1~19.8	10.1	more rounded
Amylomaize VII	6.1~14.8	9.1	elongated appendages

sodium chloride [$(C_6H_{12}O_6)_2 \cdot H_2O \cdot NaCl$] crystals identified by #39-1565 from the diffraction pattern search library (data not shown).

Crystalline characteristics by X-ray diffractometry

X-ray diffractometry was used to follow structural changes of starches during the formation and isolation of RS. While native corn starch showed an A-type X-ray profile as indicated by four strong peaks between 2θ 15.0 and 23.5° typical in cereal starches, Amylomaize V and VII starches exhibited a combination of B- and V-type crystalline structures (20): The

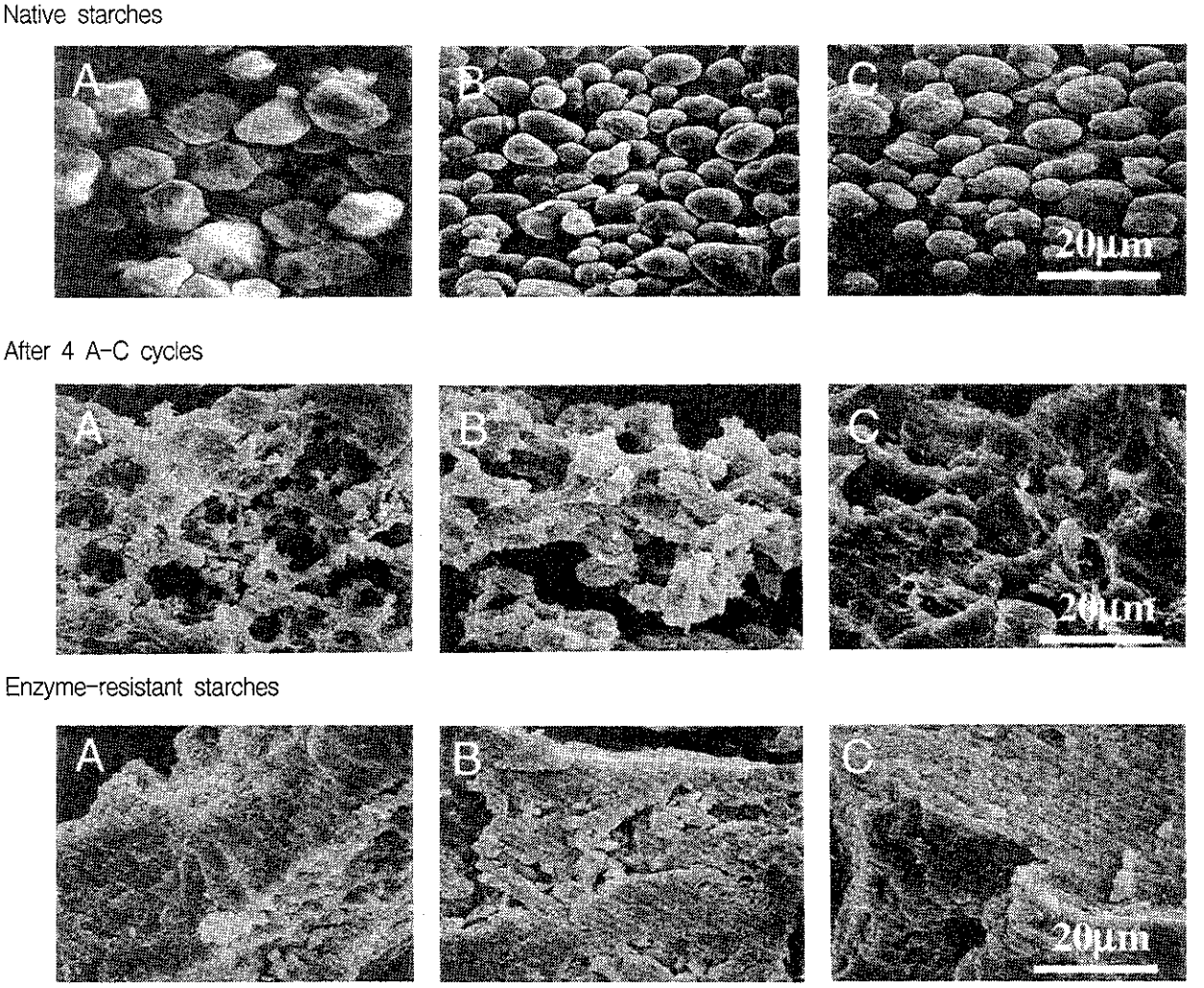

Fig. 1. Scanning electron micrographs of native starches, after four autoclaving-cooling cycles, and of enzyme-resistant starches from regular corn (A), Amylomaize V (B), and VII (C).

unique small peak at 2θ 5.6°, a strong reflection at 2θ 17.2°, and a poorly resolved doublet around 22.0~24.0° are typical diffraction lines for the B-type structure. The peak at 2θ 19.8° can be interpreted as a V-type due to amylose-lipid complexes (Fig. 2). Regular corn starch lost most of its crystallinity during autoclaving, but the crystallinity still remained in Amylomaize V and VII starches as diffuse or poor B-types (as identified by peaks around 2θ 5.5°, ~17° and ~22.5°, Fig. 3). During isolation of RS, the crystalline fraction is concentrated, so a more pronounced X-ray diffraction pattern was obtained for the isolated RS. Similar poor B-types, which is generally found for RS and retrograded starch (7,17,21-23), were obtained in all RS residues regardless of amylose contents (Fig. 4).

The typical A-type reflections in native regular corn starch

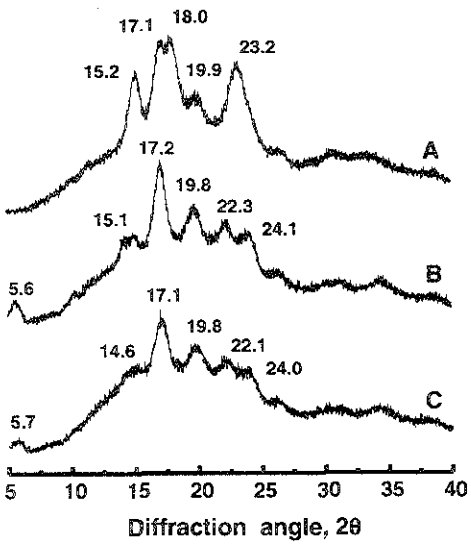

Fig. 2. X-ray diffractogram of native starches from regular corn (A), Amylomaize V (B), and VII (C).

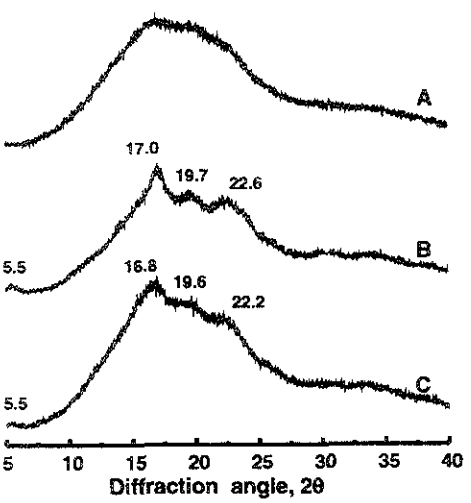

Fig. 3. X-ray diffractogram of starches after four autoclaving-cooling cycles from regular corn (A), Amylomaize V (B), and VII (C).

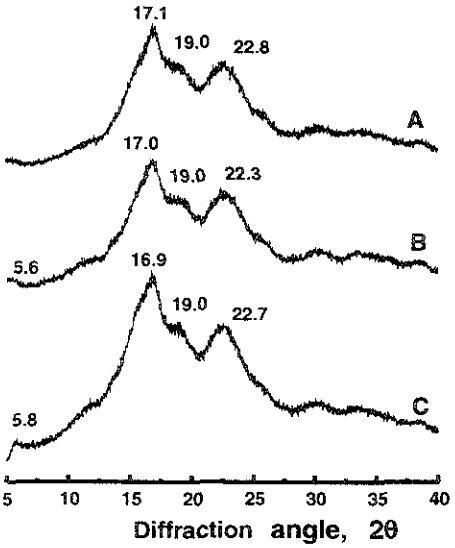

Fig. 4. X-ray diffractogram of enzyme-resistant starches from regular corn (A), Amylomaize V (B), and VII (C).

and V-type structures in native amylomaize starches disappeared, and a poor B-type pattern emerged during repeated heating and cooling. A structural transformation of regular corn starch might be due to increasing recrystallization of amylose-chain-double helices. During the retrogradation process of amylose solution with a concentration higher than 1.5%, phase separation occurs in the first step resulting in a continuous network of the polymer-rich phase; in the second step, double helices are formed in the polymer-rich phase and these aggregate to form a three-dimensional crystalline structue of the B-type (2). Loss of V-type structures in amylomaize starches could be regarded as hydrolysis of less resistant amylose-lipid complexes by amylolytic enzymes during isolation of RS residues since the peak at 2θ 19.8° became smaller in autoclaved starches (Fig. 3) and finally disappeared after enzyme hydrolysis (Fig. 4). The broad diffraction lines (Fig. 3, 4) strongly suggest that smaller and/or imperfect crystallites are present in RS than in native starch, where as the sharp, well resolved pattern (Fig. 2) reflected a higher degree of crystallite perfection.

Thermodynamic characteristics by DSC

Transition temperatures and enthalpy of native starches were higher in Amylomaize V and VII starches than those of regular corn starch (Fig. 5 and Table 3). Regardless of amylose contents, all RS residues exhibited endothermic transitions over a similar temperature range (135~169°C), with a mean peak temperature of ~154°C, which is generally found for retro graded amylose crystallites (Fig. 5 and Table 3). RS type III is thermally very stable. When isolated RS is heated in the presence of water, an endotherm is revealed in the 120~165°C temperature range with a peak transition around 155°C and with enthalpy values varying 8 to 30 J/g (2,3,13,23). Higher transition

Table 3. Thermodynamic characteristics of native starches and enzyme-resistant starches[1,2]

Type of starch		Transition Temperatures			Enthalpy
		To	Tp	Tc	ΔH
Native	Regular corn	67.15±0.32	71.15±0.58	80.94±0.20	11.22±0.04
	Amylomaize V	73.33±0.65	93.26±2.50	104.04±1.83	15.62±0.82
	Amylomaize Ⅶ	70.20±0.89	92.99±2.95	104.04±2.58	16.36±1.16
RS	Regular corn	143.82±0.80	156.23±0.42	168.65±0.57	27.42±4.10
	Amylomaize V	134.60±1.15	151.87±0.65	165.14±0.87	25.60±0.80
	Amylomaize Ⅶ	142.92±1.04	155.17±0.90	165.34±0.75	26.58±4.50

[1]Values are expressed as mean±stardard error.
[2]To, Tp, and Tc mean onset, peak, and completion transition temperatures (°C), respectively. ΔH means transition enthalpy (J/g).

Table 4. Relationship between PHI values and RS yield from regular corn, Amylomaize V, and Ⅶ starches

Type of starch	Amylose (%)	RS yield (%)	Transition Temperatures (°C)			Enthalpy (J/g)	PHI[1]
			To	Tp	Tp-To	ΔH	
Regular corn	25.9	14.5	67.15	71.15	4.00	11.22	2.81
Amylomaize V	46.6	27.7	73.33	93.26	19.93	15.62	0.78
Amylomaize Ⅶ	67.0	36.1	70.20	92.99	22.79	16.36	0.72

[1]PHI: Peak Height Index [ΔH/(Tp-To)]

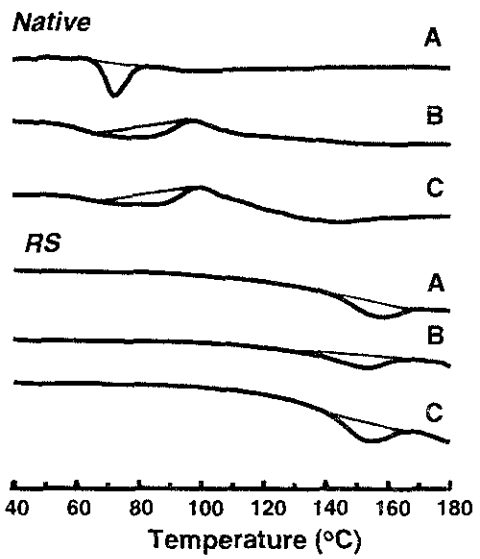

Fig. 5. DSC thermogram of native starches and enzyme-resistant starches from regular corn (A), Amylomaize V (B), and Ⅶ (C).

temperature, enthalpy, and RS yield of Amylomaize V and Ⅶ starches were related to the granular stability shown by the microscopic (Fig. 1) and crystallographic (Fig. 3) studies after four autoclaving-cooling cycles.

The formation of enzyme-resistant fractions in gelatinized starch, containing both amylose and amylopectin, could be due to retrograded amylose and/or amylopectin. Therefore, it seems logical to assume that low RS yields are obtained when more severe enzyme hydrolysis conditions are applied, i.e. higher incubation temperatures, longer times, and higher enzyme levels. When applying incubation temperatures up to 100°C, starches gelatinize and hence no quantification of RS type Ⅱ is made. Furthermore, retrograded amylopectin, which exhibits a melting temperature in the range of 55~70°C, and amylose-lipid complexes, with melting temperatures in the range of 90~110°C, are easily hydrolysed when incubated with heat-stable α- amylase at 100°C. Also proteolytic enzymes are used in a pre-treatment step in order to set physically inacessible starch (Type Ⅰ RS) free or to remove the amylolytic enzymes used in the determination (2). Since heat-stable α-amylase, protease, and amyloglucosidase were used in this study, RS residues isolated can be considered as crystallization of amylose (Type Ⅲ RS) in a partially crystalline polymer system, which was confirmed by the B-type crystalline structure and single endotherm around 155°C with an enthalpy value of 27 J/g.

Peak height index (PHI), the ratio of ΔH to Tp-To calculated from DSC data, varies directly with ΔH and inversely with the temperature range of the gelatinization. It provides a numerical value that is descriptive of the relative shape of the endotherm, e.g., a tall narrow endotherm has higher PHI than does a short broad one, even if the energy involved in the transformation is the same (24). Since PHI was strongly correlated to RS yield (r=-0.932) and collection of DSC data is much easier and takes less time than measurement of amylose content, it may be a good index to predict RS yield. PHI decreased as amylose content increased: 2.81 for regular corn, 0.78 for Amylomaize V, and 0.72 for Amylomaize Ⅶ (Table 4).

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