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Note

Effect of aliphatic hydrocarbon groups on the crystallization of amylodextrin: model experiments for starch crystallization

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To elucidate the mechanism of the formation of crystalline polymorphs of starch granules (A-, B-, and C-types), the effect of aliphatic alcohols and acids on the crystallization of Naegeli-type amylodextrin (resembling Naegeli amylodextrin prepared from potato starch) was studied. Addition of the alcohols and the acids to the crystallization media for the amylodextrin shifted the resulting structure towards the A-type from the B-type. The extent of the shift was greater with an increase of both the molecular size and the concentration of the additive. Decanoic and myristic acids (1 mg/ml) shifted the crystallization of the amylodextrin from the Cb-type to the Ca-type and to the A-type, respectively. Lysolecithin was a little less effective than the component fatty acid. The marked effects of free fatty acid and lysolecithin, which are abundant lipids of cereal starches, suggest that these lipids may play an effective role in the *in vivo* formation of the A-type of cereal starches.

INTRODUCTION

Three kinds (A-, B-, and C-types) of crystalline polymorphs are known in native starch granules[1]. The A- and B-types differ clearly, but the C-type appears to be a series of mixtures of the A- and B-types in various proportions[2]. Hizukuri *et al.*[3] reported that the crystalline structure of soybean-seedling starch alters with the growth temperature; a higher temperature tends to shift the structure toward the A-type. Similarly, sweet potato is affected by soil temperature[4]. On the other hand, rice[5], potato[5], and chlorella[6] produce their own characteristic types, independent of their growth temperatures. These findings suggest that both the environmental temperature and the physiological conditions inside cells are concerned in the determination of the polymorphs. The previous studies[7,8] on the crystallization of amylodextrin, the model for the crystallization of starch, suggested that the small ions inside cells are concerned in the formation of the polymorphs. We have now examined the effects of lipids and alcohols on the crystallization of amylodextrin,

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because cereal starches of the A-type have more lipid than starches of the B- and C-types. The results suggest that lipid may play an effective role in the *in vivo* formation of the A-type.

RESULTS

The conditions for the crystallization of Naegeli-type amylodextrin (amylodextrin-NT) were examined in regard to the temperature and the concentration, to determine the optimal conditions for the subsequent studies. The results are summarized in Table I. Amylodextrin-NT, crystallized from 35 and 50% (w/v) aqueous solutions in the temperature ranges 24.5–29.5 and 23–28°, respectively, showed a series of structural changes from the B- to the A-types. The trends of the shift with temperature and concentration were similar to those found in a previous study[9], but

TABLE I

RELATIONSHIP BETWEEN THE CONDITIONS FOR CRYSTALLIZATION OF AMYLODEXTRIN-NT, TEMPERATURE, AND CONCENTRATION, AND THE CRYSTALLIZING POLYMORPHS[a]

Amylodextrin concentration (%)	*Temperature (degrees)*								
	23.0	*24.5*	*25.5*	*26.0*	*27.0*	*27.5*	*28.0*	*28.5*	*29.5*
35	B	B	Cb	Cb	Cb	Cc	Ca	Ca	A
50	B	Cb		Cc	Ca		A		A

[a]The C-type is divided into three subgroups (Ca, Cb, and Cc) according to the closeness of the X-ray diffraction pattern to those of the A- and the B-types, and to the intermediate between them[7].

the temperature for the shift was higher than that previously reported (*e.g.*, 15–20° at 35.1%). The higher molecular weight of amylodextrin-NT than the previous amylodextrin ($\overline{d.p.}$ 12.6) may raise the shifting-temperature, but this is not clear.

Figs. 1 and 2 show the effect of the size of hydrocarbon groups of monohydric alcohols on the crystallization. Amylodextrin-NT, which crystallized into the B-type at 22° from 35% aqueous solution, gave a series of the C-type changing progressively towards the A-type in the presence of a series of alcohols (35 mg/ml) in which the size of the hydrocarbon group increased from methyl to butyl, as shown in Fig. 1. The effects of 1-pentanol and 1-hexanol were compared in 2M methanolic solution, because of their low solubilities in water (Fig. 2). The enhancing effect on shifting the crystallization towards the A-type with the increase in the size of the hydrocarbon group was thus observed clearly with aliphatic alcohols. The extents of the effects of butyric acid and 1-butanol were the same, as shown in Fig. 3, and a similar size-effect of the hydrocarbon group was observed in the same series of monocarboxylic acids. Decanoic and myristic acids, at low concentrations (1 mg/ml), shifted the crystallization from the Cb- to the Cc- and the A-types, respectively (Fig. 4). A lysolecithin, 1-palmitoyl-3-glycerophosphoryl-choline, which is an abundant lipid

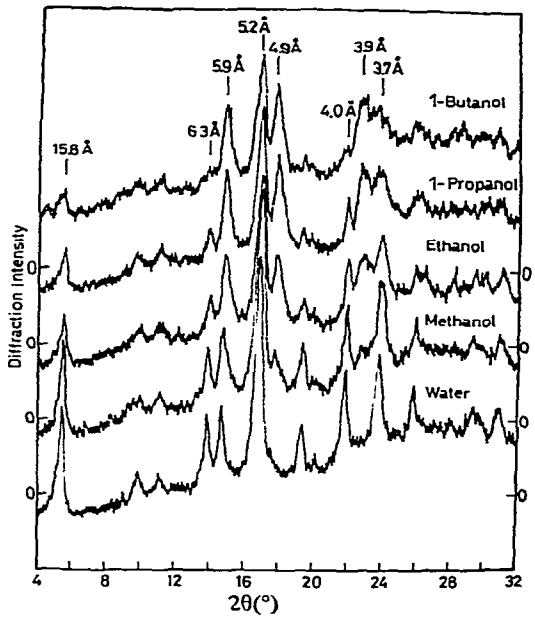

Fig. 1. X-Ray diffraction patterns of amylodextrin-NT crystallized in the presence of aliphatic alcohols (C_1–C_4). Conditions: temperature, 22°; amylodextrin concentration, 35% (w/v); alcohol concentration, 35 mg/ml; medium, water. The X-ray diffraction patterns are the B- (water, control), the Cb- (methanol and ethanol), the Cc- (1-propanol), and the Ca-types (1-butanol), respectively; for the A-type, see Fig. 5, 10%.

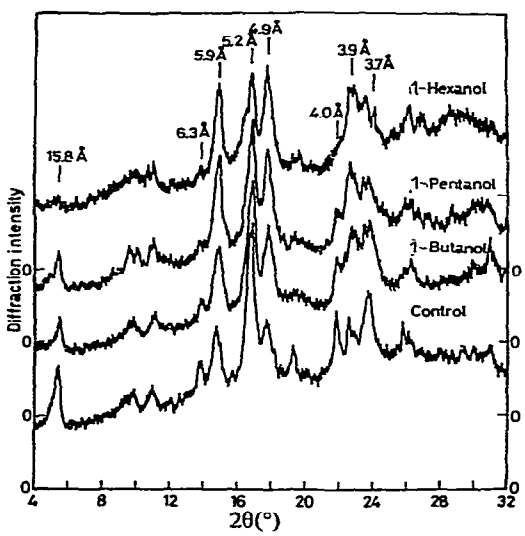

Fig. 2. X-Ray diffraction patterns of amylodextrin-NT crystallized in the presence of aliphatic alcohols (C_4–C_6). Conditions: temperature, 20°; amylodextrin concentration, 35% (w/v); alcohol concentration, 10 mg/ml; medium, 2M methanol.

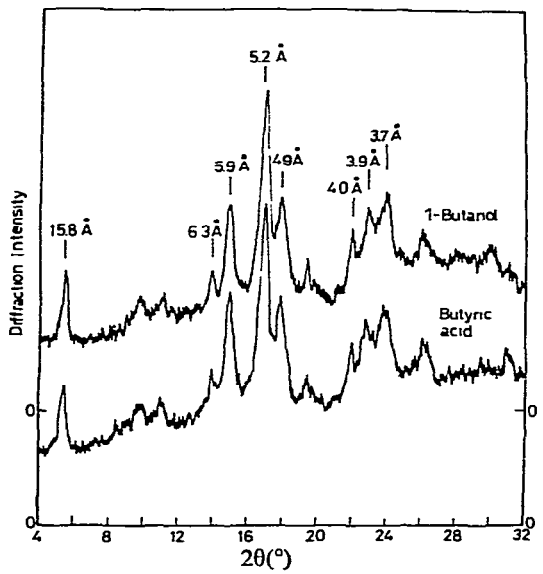

Fig. 3. Comparison of the effects of 1-butanol and butyric acid on the crystallization of amylodextrin-NT (X-ray diffraction patterns). Conditions: temperature, 20°; amylodextrin concentration, 50% (w/v); 1-butanol and butyric acid concentrations, 12.5 mg/ml; medium, water. The control produced the B-type.

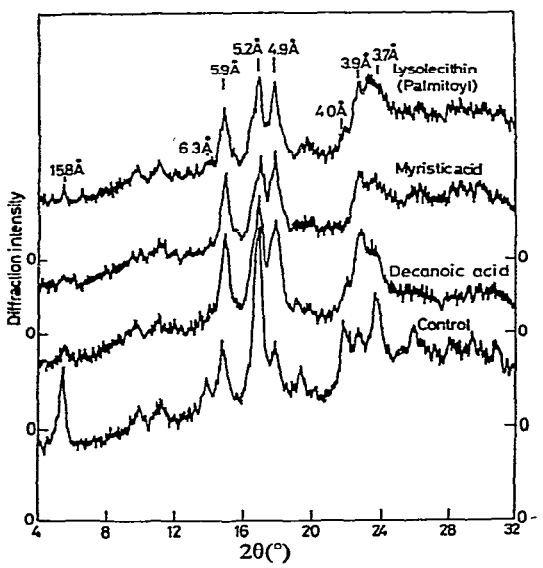

Fig. 4. X-Ray diffraction patterns of amylodextrin-NT crystallized in the presence of lipids. Conditions: temperature, 13.5°; amylodextrin concentration, 35% (w/v); concentrations of decanoic and myristic acids, 1 mg/ml; concentration of lysolecithin, 1.93 mg/ml (palmitoyl content, 1 mg/ml); medium, 0.5M potassium phosphate buffer (pH 6.9) containing 2M ethanol.

of wheat starch[10,11], was a little less effective than myristic acid, but was still a potent effector (Fig. 4). In addition, it may play an effective role in enlarging the shift *in vivo* by solubilizing other insoluble lipids.

The effect appears to be independent of the positions of the hydroxyl group and the branching of the carbon chain of alcohols, since the isomeric butyl alcohols (*n-*, *sec-*, *iso-*, and *tert-*) all gave the same extent of shift towards the A-type under the optimal conditions for the comparison.

In general, the effect was enhanced by an increase of concentration, as observed for inorganic ions[7]. Production of the B-type from an aqueous solution was transformed to that of the Ca- and A-types in the presence of 5 and 10% of 1-butanol, respectively, as shown in Fig. 5. It is of interest that the A-type, but not helical inclusion complexes, was produced in the presence of butanol and fatty acids. Conceivably, this situation is due to the short unit-chains of the amylodextrin.

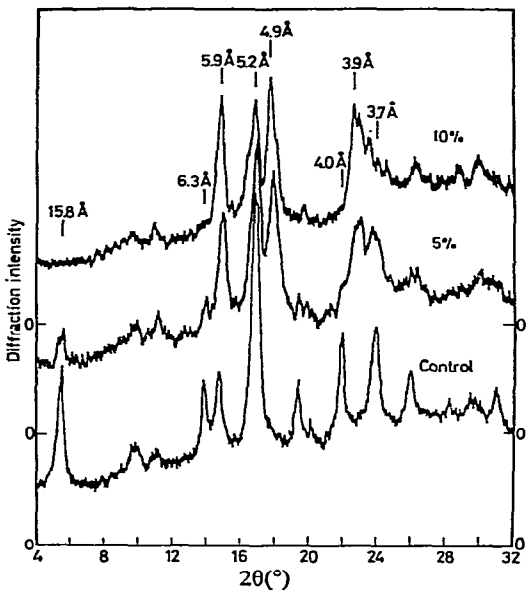

Fig. 5. Effect of the concentration of 1-butanol on the crystallization of amylodextrin-NT (X-ray diffraction patterns). Conditions: temperature, 20°; amylodextrin concentration, 50% (w/v); medium, water.

Besides lipids, some amino acids, organic acids of the tricarboxylic acid cycle, and sugars also affected the shift (toward the A- from the B-type) under certain conditions, and the extents of the shifts caused by these compounds and lipids were compared. The results described below clarified the superior effect of lipids to those of other materials tested. With 35% (w/v) amylodextrin in 1.5M potassium phosphate buffer at 25°, additions of 5 mg/ml of decanoic acid and 1-palmitoyl-glycerophosphoryl-choline shifted the crystallization from the Cb- to the Ca-type, whereas the addition of 10 mg/ml of the following compounds gave no or only a little shift: 13 amino acids (Ala, Arg, Asp, Asn, Cys, Glu, Gln, His, Leu, Met, Pro, Ser, and

Try); citric, succinic, fumaric, and malic acids; D-glucose and D-fructose; and sodium and potassium chloride.

DISCUSSION

Most cereal starches are of the A-type and contain ~1% of lipid, whereas most other starches are of the B- or C-types and contain ~0.1% of lipid. Corn starch contains 0.8% of lipid, of which the main components[10] are free fatty acid (62%) and lysolecithin (18%). Wheat starch contains ~1% of lipid, of which more than 60% is lysolecithin[10–15]. In general, the main components of lipid in cereal starches are free fatty acid and lysolecithin. The reason for the presence of these lipids in these starches is not clear. Possibly, cereal starches are synthesized in amyloplasts containing a high concentration of these lipids, which are ready to form inclusion complexes with newly formed amylose. The present results suggest that soluble lipids act to form the A-type starch *in vivo*. The B-type of amylomaize starch, which has more lipid than normal corn-starch[12], cannot be explained in this way. The reason may be because the high amylose content of amylomaize starch is capable of including more lipid and thus of lowering the lipid level inside the amyloplast membrane. Another possibility is that amylomaize may contain substances that cause the reverse shift from the A- to the B-type, although only SO_4^{2-} has been reported to do this[7].

Hydrocarbon groups in an aqueous solution of amylodextrin may cause less hydration of amylodextrin through the interactions between hydrocarbon groups, water, and amylodextrin, and may lead to crystallization of the A-type, which has been suggested to have less water of crystallization than the B-type[16].

EXPERIMENTAL

Preparation of amylodextrin-NT. — Amylodextrin-NT (resembling Naegeli amylodextrin) was prepared by a rapid process of steeping potato starch in M HCl–70% ethanol at 65° for 48 h, as described previously[17]. The resulting amylodextrin was washed repeatedly with 70% ethanol by suspending and centrifuging. The aqueous solution of the amylodextrin (~8%), which was slightly turbid and acidic (pH 3–4), was neutralized to pH 6–7 by addition of Dowex-50 (HO^-) resin, centrifuged at 10,000*g* for 10 min, and then lyophilized. The resulting amylodextrin was easily soluble in hot water and gave a clear and concentrated solution. The $\overline{\text{d.p.}}$ values per reducing and non-reducing end were 21.0 and 10.5, respectively. The reducing end was determined by the method of Somogyi, using the colorimetric reagent of Nelson, but the specimen was heated with Somogyi reagent for 30 min at 100° as described previously[18]. The non-reducing end was determined by rapid Smith-degradation[19]. Total carbohydrate was determined[20] colorimetrically with anthrone–H_2SO_4.

Crystallization of amylodextrin. — A suspension of amylodextrin-NT (0.7–1.0 g,

dry weight) in water or an appropriate buffer (0.7–1.0 ml) was heated in a boiling-water bath until a clear solution was obtained (~1.5 min), and then cooled to the desired temperature for crystallization. To the clear solutions, appropriate amounts of alcohols or lipids were added. Each mixture was made up to 2.0 ml with water or the buffer, and incubated at the desired temperature for 48 h. The amylodextrin crystallized, and was collected on a glass filter under reduced pressure and washed with 2 ml of water at the desired temperature.

Conditions for X-ray diffraction. — The foregoing, wet amylodextrin was packed into a frame and subjected to X-ray irradiation. The X-ray diffraction apparatus (Rigakudenki Co., Model 17-3P) was operated under the following conditions: high-tension voltage, 30 kV; current, 12.5 mA; scanning speed, 1°/min; chart speed, 10 mm/min; time constant, 4 sec; divergence and receiving slits, 1°; X-rays, CuKα, eliminating Kβ with a nickel filter.

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