ELSEVIER

PII: S0144-8617(97)00022-2

Carbohydrate Polymers 33 (1997) 187–194

0144-8617/97/$17.00 + .00

Some properties of branched and linear dextrins from Nägeli amylodextrin

Kanefumi Kitahara, Emi Eitoku, Toshihiko Suganuma and Tomonori Nagahama

Department of Biochemical Science and Technology, Faculty of Agriculture, Kagoshima University, 1-21-24, Korimoto, Kagoshima 890, Japan

(Received 7 June 1996; revised version received 14 August 1996; accepted 15 September 1996)

Branched dextrin (BD) and linear dextrin (LD) were prepared from Nägeli amylodextrin, and their molecular structure and some properties (the interaction with 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) in solution and the aggregation into precipitate) were investigated. BD and LD were singly branched dextrin (d.p. = 25.7) with the branched point near the reducing end, and practically linear dextrin (d.p. = 12.4), respectively. The fluorescence intensity of TNS increased with increasing concentrations of α-cyclodextrin, BD and LD, indicating their interactions with each other, in which the values of the dissociation constant were estimated to be 20 mM, 3.4 mM and 7.8 mM, respectively. In aqueous 16 M methanol, the aggregation of BD was much faster than that of LD, and both dextrins were precipitated into a type A crystallite. It is considered that the branch point in the BD which is a connection of two linear dextrins may trigger the association of the unit chains. On the other hand, the aggregation of dextrins was retarded in the presence of lauric acid. These results may be applicable to the local phenomenon of the whole amylopectin. © 1997 Elsevier Science Ltd

INTRODUCTION

Starch is composed of a mixture, in varying proportion, of amylose, which is a predominantly linear (1→4)-α-D-glucan but contains a few α-(1→6) branch points, and of amylopectin, which is a higher molecular mass (1→4)-α-D-glucan with frequent branch points. The amylose is able to make crystalline complexes with lipids and also some chemical compounds, such as *n*-butanol, thymol and surfactants, resulting in relatively heat-stable precipitates, and these materials are commonly used for the separation of amylose and amylopectin. On the other hand, although the complexing ability of amylopectin has been confirmed in some reports (Batres and White, 1986; Eliasson and Ljunger, 1988; Gudmundsson and Eliasson, 1990, 1992; Gudmundsson, 1992) there remain some unclear points concerning the interaction of amylopectin with lipids, because of its larger molecular weight and more complicated molecular structure compared with those of amylose.

When starch granules are treated with an aqueous acid, such as 15% sulfuric acid (Nägeli, 1874) or 7.5% hydrochloric acid (Lintner, 1886), at ambient temperature, an acid resistant part of the granules remains after the amorphous parts have been eroded away. In general, the residues are termed the Nägeli amylodextrin and lintnerized starch, respectively. It was reported that these acid resistant parts were chromatographically separated into three fractions, fractions I, II and III, which are characterized as multiply branched dextrins, singly branched dextrins with the branch points near the reducing group, and linear dextrins, respectively (Watanabe and French, 1980; Umeki and Kainuma, 1981). Hall and Manners (1980) reported that fraction II contained, in addition to the singly branched dextrins, about one-third doubly branched dextrins.

In this study, the branched and linear dextrins were prepared from Nägeli amylodextrin, and their molecular structure and some properties (interaction with complexing reagents in solution and aggregation into precipitate) were investigated. It is considered that such branched dextrin is one of the simplest partial structures of amylopectin, and that the results of this study are useful for elucidation of the properties of the whole amylopectin molecule. Also, the comparison of branched dextrin with linear dextrin provides the

definitive effect of the branch point on properties of starch.

MATERIALS AND METHODS

Waxy corn starch was obtained from Nihon Shokuhin Kako Co. Ltd. (Tokyo). Nägeli amylodextrin (NA) was prepared by leaching the waxy corn starch with 15% sulfuric acid (w/w) at 35 °C (shaken by hand once a day) for 60 days until corrosion of the starch leveled off. The solubilized starch was determined by the phenol–sulfuric-acid method (Dubois *et al.*, 1956).

Alpha-cyclodextrin (α-CD, >98.5%) was obtained from Ensuiko Sugar Refining Co. Ltd. (Yokohama). All reagents and solvents, unless otherwise specified, were purchased from Wako Chemical Industries (Osaka). The solvents were freshly distilled before use.

Isoamylase originated from *Pseudomonas* sp. and 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) was purchased from Nacalai Tesque Inc. (Kyoto). Pullulanase (crystalline) from *Klebsiella pneumoniae* was purchased from Hayashibara Biochemical Laboratories Inc. (Okayama, Japan). Ammonium sulfate in both enzyme preparations was removed by gel filtration with Sephadex G-25 (1×30 cm, Pharmacia Biotech, Tokyo) using high-performance liquid chromatography (HPLC) before use. β-Amylase was purified from sweet potato (*Shiroyutaka cultivar*) by the method of Takeda and Hizukuri (1969).

Preparation of branched and linear dextrins

The NA was fractionated by the stepwise precipitation method using pyridine–methanol–water, reported by Kikumoto and French (1983) to give fractions I, II and III. In this study, fractions II and III were used as branched dextrin (BD) and linear dextrin (LD), respectively. The fractions of BD and LD were purified again by the same fractionation procedure, and crystallized twice from aqueous 70% methanol.

Determination of chemical structure of dextrins

The average degree of polymerization ($\overline{\text{d.p.}}$) and the limit of β-amylolysis were determined by the methods of Hizukuri *et al.* (1981).

The dextrins were successively debranched by isoamylase, and then pullulanase. The dextrins (25 mg, dw) were treated with isoamylase (2.5 U) in 5 ml of 60 mM sodium acetate buffer (pH 3.5) at 45 °C for 24 h. After stopping the reaction by heating, the pH of the solution was adjusted to 5.5 by adding 1 M sodium hydroxide, and then the dextrins were treated with pullulanase (17 U) at 30 °C for 24 h (final volume, 6 ml). One unit causes the formation of 1 μmol of reducing sugar (as determined by Somogyi–Nelson method (Nelson, 1944; Somogyi, 1952)) in 1 min. The reaction conditions are as follows: isoamylase, soluble starch as substrate at pH 3.5 and 30 °C; pullulanase, pullulan as substrate at pH 5.5 and 30 °C.

Gel-permeation chromatograph (GPC) analysis of debranched dextrins was carried out by the method previously reported (Kitahara *et al.*, 1994).

High-performance anion-exchange chromatography (HPAEC) was conducted using a Dionex system (Dionex, Sunnyvale, CA) under the conditions of Hanashiro *et al.* (1996), except for the composition of the eluent as follows: the percentage of 150 mM sodium hydroxide containing 500 mM sodium acetate solution was 30 at 0 min, 40 at 5 min, 60 at 25 min, 80 at 65 min and 100 at 85 min.

Reaction of dextrins with TNS

TNS was recrystallized twice from distilled water before use (McClure and Edelman, 1966). The concentration of TNS was determined spectrophotometrically, taking the molecular absorbance $\varepsilon_{TNS} = 4860$ (Kitamura, private communication).

TNS (187 μM) in distilled water (1 ml) was added to each of the BD, LD and α-CD solutions (1 ml) in 160 mM sodium acetate buffer at pH 5.3. After they had stood for 1 h at 25 °C in the dark, the fluorescence intensity was measured at 460 nm with excitation at 366 nm using a Hitachi F-2000 fluorospectrophotometer (Hitachi, Tokyo). The measurements of BD, LD and α-CD were carried out at 0–1.54 mM, 0–2.86 mM and 0–4.48 mM of the final carbohydrate concentration, respectively. Each carbohydrate concentration is expressed as a molar concentration estimated on the basis of the respective average molecular weight calculated from the $\overline{\text{d.p.}}$ The value of the dissociation constant (K_d) was determined by the least-squares method to fit the experimental results according to the equation, assuming 1:1 complex formation as reported by Nakatani *et al.* (1977).

$$D + T \leftrightarrows DT \tag{1}$$

$$K_d = [D] \cdot [T]/[DT]. \tag{2}$$

D, T and DT are dextrins (α-CD, BD or LD), TNS and the dextrin–TNS complex, respectively. $[D]$, $[T]$ and $[DT]$ show the equilibrium concentrations of dextrins, TNS and the dextrin–TNS complex, respectively, and K_d is the dissociation constant.

$$[D]_0 = [D] + [DT] \tag{3}$$

$$[T]_0 = [T] + [DT]. \tag{4}$$

$[D]_0$ and $[T]_0$ are the initial concentrations (analytical concentrations) of dextrins and TNS, respectively. The following equation is derived from eqns (2), (3) and (4):

$$[DT] = \frac{1}{2}[[D]_0 + [T]_0 + K_d - \{([D]_0 + [T]_0 + K_d)^2 - 4 \cdot [D]_0 \cdot [T]_0\}^{0.5}]. \quad (5)$$

The observed fluorescence intensity, ΔF (arbitrary units), is proportional to the concentration of DT:

$$\Delta F = f \cdot [DT] \quad (6)$$

where f is the proportionality coefficient.

$$\Delta F = \frac{f}{2} \cdot [[D]_0 + [T]_0 + K_d - \{([D]_0 + [T]_0 + K_d)^2 - 4 \cdot [D]_0 \cdot [T]_0\}^{0.5}]. \quad (7)$$

Thus, eqn (7) was used for the curve fitting, which was done by using DeltaGraph Pro 3.5 (DeltaPoint Inc., USA) on a Macintosh computer.

Aggregation properties of dextrins

The dextrins (35 mg or 70 mg, dw) were dissolved in 2.4 ml of distilled water in a boiling water bath for 3 min, and were then cooled to 25 °C in a thermostated water bath for 10 min. Methanol, with and without 35 μmol of lauric acid, was added to the solution up to a final concentration of 16 M, and it was made up to 7 ml with distilled water. The turbidity development (25 °C) was measured using a Klett–Summerson photoelectric colorimeter (Klett Manufacturing Co. Inc., USA) with a #66 filter. Multiple determinations were performed.

After the above experiment, the incorporation of the lauric acid into the aggregate was estimated from its recoverability in the supernatant after centrifugation (4500*g*, 10 min). The content of lauric acid in the supernatant was determined using an NEFA-C test kit (Kitahara *et al.*, 1994).

X-ray diffraction analysis

The X-ray diffractogram was obtained using an X-ray diffractometer (Rigakudenki RAD-RB) as previously reported (Kitahara *et al.*, 1993).

RESULTS AND DISCUSSION

Chemical structures of dextrins

In this study, firstly the molecular structures of the prepared dextrins from NA were confirmed.

The $\overline{\text{d.p.}}$ of NA and the fractionated BD and LD were estimated from their total and reducing sugar contents to be 15.5±0.9, 25.7±0.4 and 12.4±0.3 ($n = 3$), respectively, and the $\overline{\text{d.p.}}$ of LD was equal to half the $\overline{\text{d.p.}}$ of BD. The limits of β-amylolysis of BD and LD were 76% and 98%, respectively.

Figure 1 shows GPC profiles of NA, BD and LD, and those after debranching by isoamylase and pullulanase. The elution profile of NA showed two main peaks which corresponded to the fractionated BD and LD, respectively. After debranching BD, the single peak of BD was shifted to the lower fraction corresponding to the LD. It was observed that a part (8.1%) of the BD stayed at the same position, and no change was observed after even further addition of debranching enzymes, indicating that either there was a small amount of debranching enzyme resistant dextrin or there were initially longer linear chains. On the other hand, the LD was virtually unchanged after debranching. These properties of the dextrins, i.e. degree of polymerization, limit of β-amylolysis and elution profile on GPC before and after debranching, well agreed with those previously reported (Watanabe and French, 1980; Umeki and Kainuma, 1981; Kikumoto and French, 1983).

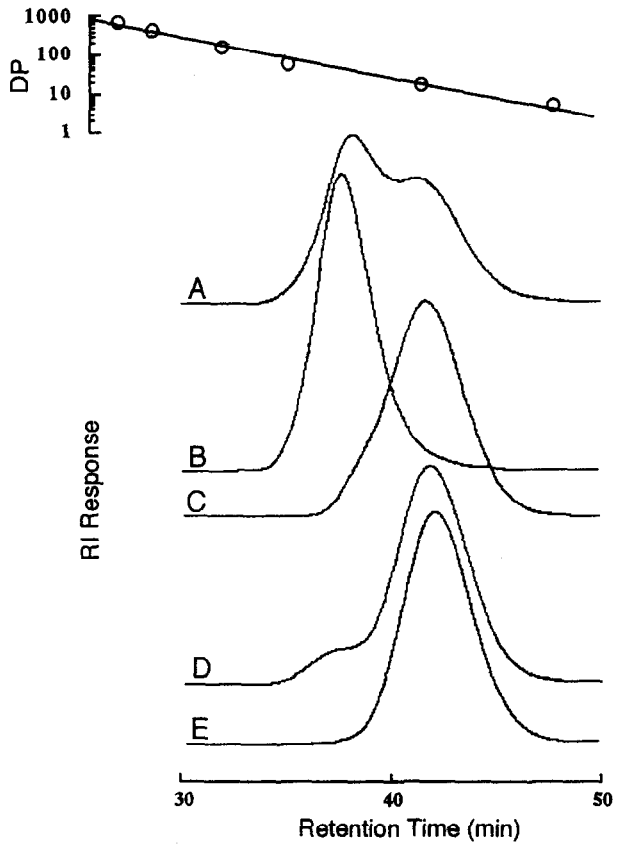

Fig. 1. GPC profiles of Nägeli amylodextrin, branched dextrin (BD) and linear dextrin (LD) fractions, and their debranched dextrins by isoamylase and pullulanase. (A) Nägeli amylodextrin, (B) BD, (C) LD, (D) debranched BD, (E) debranched LD.

In order to examine the structure more closely, the unit chain-length distribution of the dextrin was determined by HPAEC (Fig. 2). It was revealed that both dextrins were composed of similar units of chain length (mainly d.p. = 10–16). These distributions were in agreement with the previous results shown by paper chromatography (Umeki and Kainuma, 1981). It was

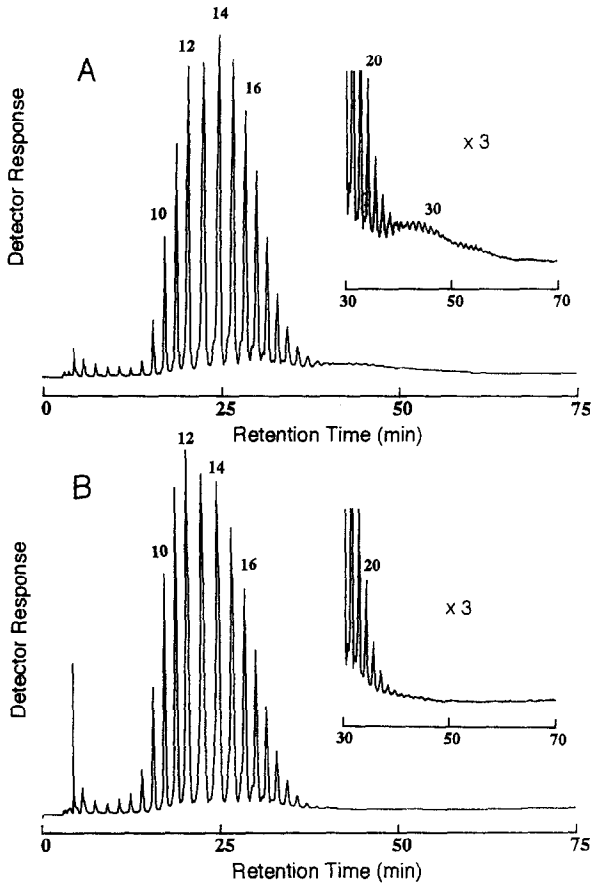

Fig. 2. HPAEC of debranched dextrins. (A) debranched BD, (B) debranched LD. Arabic numerals in the figure show the degree of polymerization.

also observed that the BD fraction included a slight amount of longer chains with d.p. > 25. On both chromatograms, peaks were found with a shoulder on the left side, indicating the existence of dextrins with branched points (Koizumi *et al.*, 1989; Rani *et al.*, 1992) even after treatment by debranching enzymes. Although the structures of these branched dextrins were not identified, they might have glucosyl stubs resulting from heterogeneous acid hydrolysis (Umeki and Kainuma, 1981), because the branched glucose cannot be hydrolysed by the debranching enzymes (Kainuma *et al.*, 1978).

In spite of the presence of branched points in most of the BD molecules, the relatively high limit of β-amylolysis (76%) suggests that the branch points in BD are located close to the reducing group of the molecule. In practice only trace amounts of large dextrins (> G8) in the β-amylase digest of BD were detected on the thin layer chromatogram (data not shown). Also, the main unit chain length of BD was about half the whole $\overline{d.p.}$ Therefore, it is concluded that the primary structure of the dextrins in BD is the singly branched dextrin with the branch point near the reducing end, previously described by Umeki and Kainuma (1981), and that some of them may have glucosyl or short chain stubs. On the other hand, the primary structure of the dextrins in LD is practically linear dextrin. Hall and Manners (1980) reported that one-third of the molecules in the branched dextrin fraction (corresponding to fraction DFII in their paper) were doubly branched dextrins; this was derived from the methylation analysis. It is probable that the short chain stubs led to this result via the end-group analysis.

Reaction of dextrins with TNS in solution

TNS is shown as a fluorescent probe for complexation or intertwinement with cyclodextrins (Kondo *et al.*, 1976), oligosaccharides (Nakatani *et al.*, 1977) and amylose (Kitamura *et al.*, 1984). Figure 3 shows the fluorescence intensity of TNS with α-CD, BD and LD as a function of the dextrin concentration. The fluorescence intensity of each dextrin increased with increasing dextrin concentration, suggesting the interaction of the dextrins with TNS.

The values of the dissociation constant (K_d) were determined. The best fit of eqn (7) in the experimental results for α-CD, BD and LD gave the values of $K_d = 20$ mM and $f = 6.9 \times 10^4$, $K_d = 3.4$ mM and $f = 2.1 \times 10^5$, and $K_d = 7.8$ mM and $f = 1.4 \times 10^5$, respectively. The K_d of α-CD was larger than that of BD and LD, suggesting lower affinity of α-CD for TNS than for BD and LD. It is interesting to note that the K_d of BD was roughly half that of LD. Ohnishi and French (1987) have studied the thermodynamics of the binding of iodine to dextrins from NA. They concluded that the unit chains of branched dextrin (corresponding to fraction II in the paper (Ohnishi and French, 1987)) individually bound the iodine molecule just as linear dextrin (corresponding to fraction III in the paper) in solution. Therefore, it is considered that the apparent high affinity of BD with TNS, assuming 1:1 complex formation, may be due to its molecular structure having two unit chains per molecule.

Aggregation properties of dextrins

In aqueous methanol, BD and LD were easily precipitated as crystallite (Kikumoto and French, 1983). As seen in Fig. 4, both precipitates showed type A on the diffractogram. The peaks of LD were sharper than those of BD, suggesting the development of large crystallite. It is interesting that the diffractogram of BD was quite similar to that of waxy corn starch.

The aggregation of glucan chains is one of the important phenomena during multifactorial events of starch retrogradation. Figure 5 shows turbidity developments of BD and LD at 0.5% and 1.0% concentrations in aqueous 16 M methanol. From their structural features mentioned above, the total number of unit chains of BD would be equal to the total

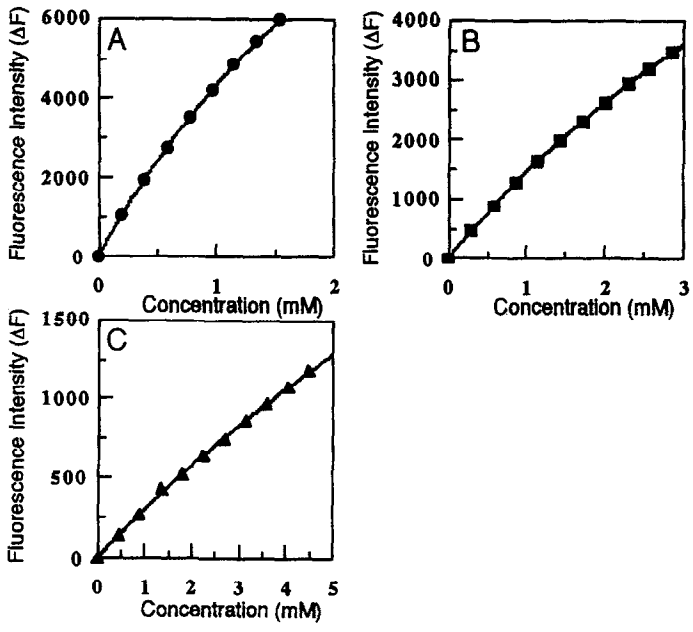

Fig. 3. Fluorescence intensity of TNS with dextrins as a function of the dextrin concentration. (A) BD, (B) LD, (C) α-CD. Solid lines are the theoretical lines obtained from a least-squares fit between the calculated and observed fluorescence intensities using eqn (7) in the text.

number of chains of LD at the same concentration. At the end of the experiments at 1.0% concentration of dextrin, 90% of BD and 74% of LD were precipitated as crystallite. The turbidity of BD developed more rapidly than that of LD, which indicated that the rate of aggregation of BD was much faster than that of LD. When the concentration was reduced to one-half its 1.0% concentration, both turbidity developments were delayed but the decrement in the case of BD was smaller than for LD. Although the aggregation rates of linear amyloses depend on their d.p. (Pfannemüller *et al.*, 1971; Gidley and Bulpin, 1989), the concentration dependence of LD on the rate was similar to that of the longer linear amylose ($\overline{d.p.} = 172$; Kitamura *et al.*, 1994). It is, therefore, considered that the difference in the concentration dependences between BD and LD is reflected in the existence of a branch point which is a connection of two linear chains near the reducing end, rather than in the difference in their $\overline{d.p.}$

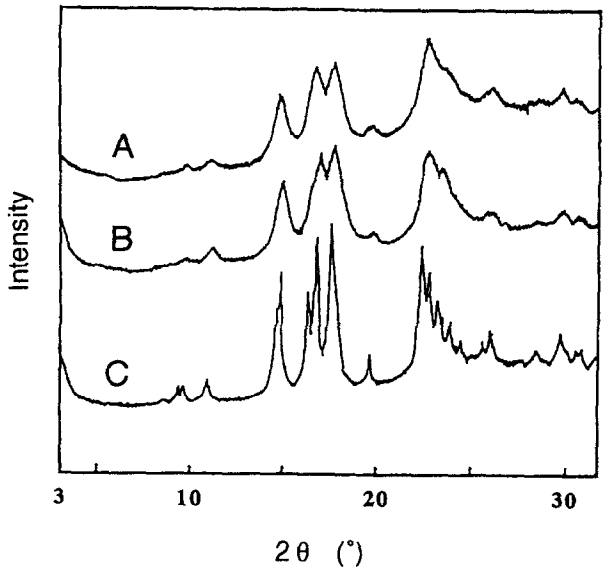

Fig. 4. X-ray diffractograms of native waxy corn starch and aggregates of dextrins in aqueous methanol solution. (A) waxy corn starch, (B) BD, (C) LD.

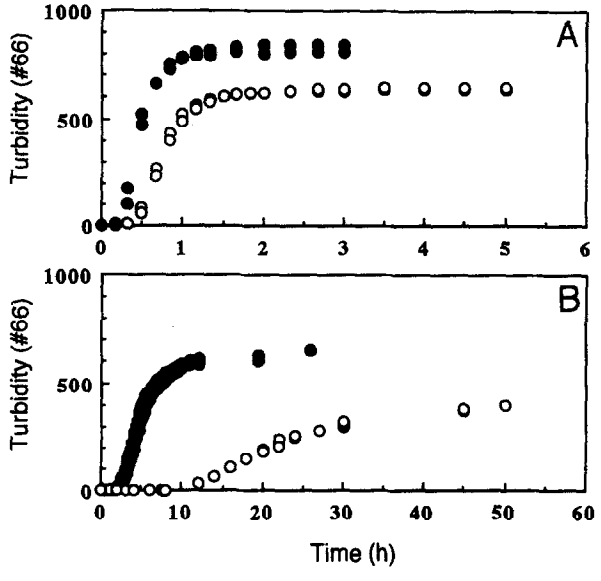

Fig. 5. Turbidity development of dextrins in aqueous 16 M methanol. (A) BD, (B) LD. Closed circles, 1.0% concentration; open circles, 0.5% concentration.

Previously, it was observed that the retrogradation of amylopectin was decreased in the presence of monoacyl lipids and surfactant (Batres and White, 1986; Eliasson and Ljunger, 1988; Gudmundsson and Eliasson, 1990, 1992; Gudmundsson, 1992). In these papers, it was declared that the formation of an inclusion complex of the amylopectin molecule with such lipids caused the retardation of retrogradation.

In this study, the effects of lauric acid on aggregation of BD and LD were investigated. As seen in Fig. 6, the turbidity developments of both BD and LD were retarded by adding lauric acid. This result indicates that, even with dextrins of simple structure, lauric acid causes depression of aggregation. This phenomenon may contribute to the retardation of retrogradation of the whole amylopectin, because such a simple structure corresponds to the outer chains of the amylopectin. It was interesting to note that the depression was less evident in the case of BD than for LD. This may indicate the characteristic property of the branch point as a trigger of dextrin aggregation.

Although the dextrins seem to interact with complexing reagents in the solution as shown by NMR study (Jane and Robyt, 1985), and also with TNS and lauric acid as shown in this study, the incorporation of lauric acid into the precipitate was estimated to be zero because all of the lauric acid added was recovered in the supernatant. Also, the X-ray diffractograms of precipitates in the presence of lauric acid were the same as those in its absence (A types, data not shown). It is concluded, therefore, that the short chain dextrins, such as the BD and LD used here, interact with lauric acid to some extent in solution, but they fail to form insoluble complexes with lauric acid. Kitamura and Kuge (1989) proposed that amylose retrogradation proceeds through a two-step process: formation of nuclei and further growth of the nuclei. From the nature of type A crystallite (Imberty *et al.*, 1988) and the fact that the BD and LD crystallized into A types, the aggregation is considered to proceed through intertwinement of two-unit chains—intramolecularly in BD and intermolecularly in LD–into double helices, followed by their arrangement. Figure 7 illustrates the possible process of the aggregation of BD in the presence of lauric acid. In solution, there are the glucan–lauric-acid interaction and the glucan–glucan interaction. Finally, the double-helical units resulting from glucan–glucan interaction precipitate into type A crystallite. Thus, the retardation of aggregation of the dextrins in the presence of lauric acid might be due to competitive interactions between the glucan–lauric-acid interaction and the glucan–glucan interaction in solution.

In order to overcome the difficulties of elucidating the properties of starch, arising from its complicated molecular structure, enzymatically (Kitamura, 1996) and chemically (Motawia *et al.*, 1995) designed simple models for starch components have been used to clarify the properties of the whole starch molecules. The singly branched dextrin is also considered to be a simple model for starch molecules, especially amylopectin.

In this study, the molecular structures of BD and LD previously proposed by Umeki and Kainuma (1981) have been reconfirmed, and their properties of interaction with complexing reagents and aggregation were studied. The results may be applicable to the local phenomenon of the whole amylopectin.

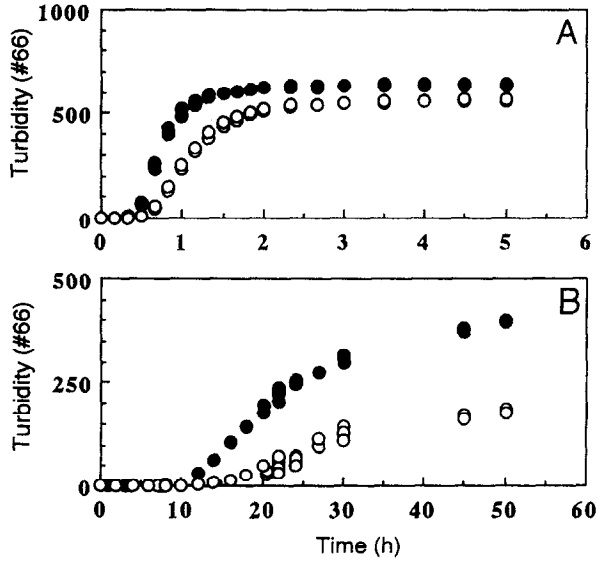

Fig. 6. Effect of lauric acid on aggregation of dextrins at 0.5% concentration in aqueous 16 M methanol. (A) BD, (B) LD. Closed circles, non-addition; open circles, 5 mM lauric acid.

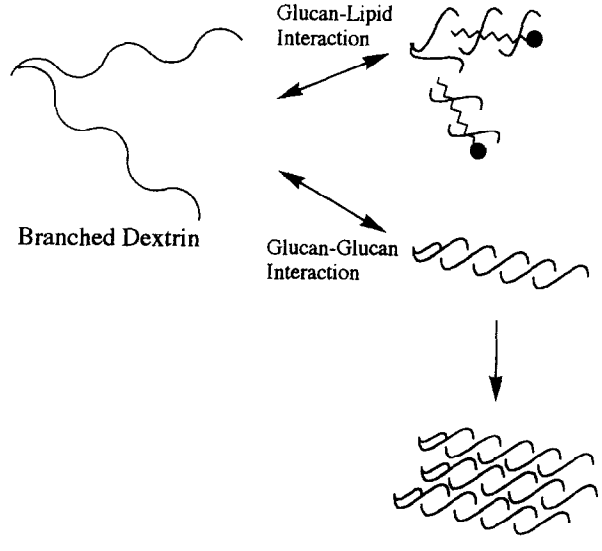

Fig. 7. Possible process of aggregation of BD in the presence of lauric acid.

Previously, we reported that fatty acids could be artificially introduced to some extent into granular NA from various starches, including waxy corn starch prepared by exhaustive treatment with 15% sulfuric acid (Kitahara *et al.*, 1993; Kaneda *et al.*, 1996). This result indicates that there is a spatial capacity for the fatty acids in the NA. In this study, however, no incorporation of lauric acid into the aggregates was observed during the aggregation of its constituting BD and LD. The structure of acid resistant regions in native starch granules might be slightly different from the ordered structure in simple models. Thus, this study provide helpful information concerning not only the local properties of the whole amylopectin molecule, but also the granular structure of native starch.

ACKNOWLEDGEMENTS

We thank Professor S. Hizukuri for the use of an HPAEC system, and Mr I. Hanashiro for his technical assistance.

REFERENCES

Batres, L.V. and White, P.J. (1986) Interaction of amylopectin with monoglycerides in model systems. *J Amer. Oil Chem. Soc.* **63,** 1537–1540.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28,** 350–356.

Eliasson, A.-C. and Ljunger, G. (1988) Interactions between amylopectin and lipid additives during retrogradation in a model system. *J. Sci. Food Agric.* **44,** 353–361.

Gidley, M.J. and Bulpin, P.V. (1989) Aggregation of amylose in aqueous systems: the effect of chain length on phase behavior and aggregation kinetics. *Macromolecules* **22,** 341–346.

Gudmundsson, M. and Eliasson, A.-C. (1990) Retrogradation of amylopectin and the effects of amylose and added surfactants/emulsifiers. *Carbohydr. Polym.* **13,** 295–315.

Gudmundsson, M. and Eliasson, A.-C. (1992) Comparison of thermal and viscoelastic properties of four waxy starches and the effect of added surfactant. *Starch/Stärke* **44,** 379–385.

Gudmundsson, M. (1992) Effects of an added inclusion–amylose complex on the retrogradation of some starches and amylopectin. *Carbohydr. Polym.* **17,** 299–304.

Hall, R.S. and Manners, D.J. (1980) The structural analysis of some amylodextrins. *Carbohydr. Res.* **83,** 93–101.

Hanashiro, I., Abe, J. and Hizukuri, S. (1996) A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. *Carbohydr. Res.* **283,** 151–159.

Hizukuri, S., Takeda, Y., Yasuda, M. and Suzuki, A. (1981) Multi-branched nature of amylose and the action of debranching enzymes. *Carbohydr. Res.* **94,** 205–213.

Imberty, A., Chanzy, H. and Pérez, S. (1988) The double-helical nature of the crystalline part of A-starch. *J. Mol. Biol.* **201,** 365–378.

Jane, J.-L. and Robyt, J.F. (1985) ^{13}C-N.M.R. study of the conformation of helical complexes of amylodextrin and of amylose in solution. *Carbohydr. Res.* **140,** 21–35.

Kainuma, K., Kobayashi, S. and Harada, T. (1978) Action of *Pseudomonas* isoamylase on various branched oligo- and poly-saccharides. *Carbohydr. Res.* **61,** 345–357.

Kaneda, Y., Kitahara, K., Suganuma, T. and Nagahama, T. (1996) Introduction of fatty acids into low-lipid starches and their Nägeli amylodextrins. *Cereal Chem.* **73,** 244–248.

Kikumoto, S. and French, D. (1983) Naegeli amylodextrin. Large scale preparation of fractions by step-wise precipitation using organic solvents. *J. Jpn. Soc. Starch Sci.* **30,** 69–75.

Kitahara, K., Suganuma, T., Fujimoto, S. and Nagahama, T. (1993) Characteristics of introduction of fatty acids into gajutsu and teppo-yuri starches. *Starch/Stärke* **45,** 30–34.

Kitahara, K., Suganuma, T. and Nagahama, T. (1994) Bound free fatty acids in glucoamylase-digested starches from corn and sweetpotato. *Cereal Chem.* **71,** 439–443.

Kitamura, S., Matsumori, S. and Kuge, T. (1984) Study of polysaccharides by the fluorescence method. V. Interaction of 2-*p*-toluidinylnaphthalene-6-sulfonate with amylose and its related compounds in aqueous solution. *J. Inclusion Phenomena* **2,** 725–735.

Kitamura, S. and Kuge, T. (1989) Conformation and physical properties of amylose in aqueous solutions. *Food Hydrocolloids* **3,** 313–326.

Kitamura, S., Hakozaki, K. and Kuge, T. (1994) Effects of molecular weight on the retrogradation of amylose. In *Food Hydrocolloids: Structures, Properties, and Functions*, ed. K. Nishinari and E. Doi, pp. 179–182. Plenum Press, New York.

Kitamura, S. (1996) Starch polymer, natural and synthetic. In *The Polymeric Materials Encyclopedia, Synthesis, Properties and Applications*, Vol. 10, ed. J. C. Salamone, pp. 331–339. CRC Press, Florida.

Koizumi, K., Kubota, Y., Tanimoto, T. and Okada, Y. (1989) High-performance anion-exchange chromatography of homogeneous D-gluco-oligosaccharides and polysaccharides (polymerization degree $\geq$50) with pulsed amperometric detection. *J. Chromatogr.* **464,** 365–373.

Kondo, H., Nakatani, H. and Hiromi, K. (1976) Interaction of cyclodextrins with fluorescent probes and its application to kinetic studies of amylase. *J. Biochem.* **79,** 393–405.

Lintner, C.J. (1886) Studien über Diastase. *Journal für Praktische Chemie* **34,** 378–394.

McClure, W.O. and Edelman, G.M. (1966) Fluorescent probes for conformational states of proteins. I. Mechanism of fluorescence of 2-*p*-toluidinylnaphthalen-6-sulfonate, a hydrophobic probe. *Biochemistry* **5,** 1908–1919.

Motawia, M.S., Olsen, C.E., Enevoldsen, K., Marcussen, J. and Møller, B.L. (1995) Chemical synthesis of 6′-α-maltosylmaltotriose, a branched oligosaccharide representing the branch point of starch. *Carbohydr. Res.* **277,** 109–123.

Nägeli, W. (1874) Beiträge zur näheren Kenntnifs der Stärkegruppe. *Justus Liebigs Annalen der Chemie*, **173,** 218–227.

Nakatani, H., Shibata, K., Kondo, H. and Hiromi, K. (1977) Interaction of amylose and other α-glucans with hydrophobic fluorescent probe (2-*p*-toluidinylnaphthalene-6-sulfonate). *Biopolymers* **16,** 2363–2370.

Nelson, N. (1944) A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* **153,** 375–380.

Ohnishi, M. and French, D. (1987) A flow-calorimetric study of the binding of iodine to amylodextrin fractions. *Carbohydr. Res.* **161,** 257–263.

Pfannemüller, B., Mayerhöfer, H. and Schulz, R.C. (1971) Conformation of amylose in aqueous solution: optical rotatory dispersion and circular dichroism of amylose–iodine complexes and dependence on chain length of retrogradation of amylose. *Biopolymers* **10,** 243–261.

Rani, M.R.S., Shibanuma, K. and Hizukuri, S. (1992) The fine structure of oyster glycogen. *Carbohydr. Res.* **227,** 183–194.

Somogyi, M. (1952) Notes on sugar determination. *J Biol. Chem.* **195,** 19–23.

Takeda, Y. and Hizukuri, S. (1969) Improved method for crystallization of sweet potato β-amylase. *Biochim. Biophys. Acta* **185,** 469–471.

Umeki, K. and Kainuma, K. (1981) Fine structure of Nägeli amylodextrin obtained by acid treatment of defatted waxy-maize starch. Structural evidence to support the double-helix hypothesis. *Carbohydr. Res.* **96,** 143–159.

Watanabe, T. and French, D. (1980) Structural features of Naegeli amylodextrin as indicated by enzymic degradation. *Carbohydr. Res.* **84,** 115–123.