Carbohydrate Research, 198 (1990) 49–55
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE RETROGRADATION AND GELATION OF AMYLOPECTINS FROM VARIOUS BOTANICAL SOURCES

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(Received February 6th, 1989; accepted for publication, August 4th, 1989)

ABSTRACT

The distribution of the chain lengths of amylopectins from wheat, barley, maize, pea, canna, and potato starches were characterized by size-exclusion chromatography. The cereal amylopectins had the shorter average chain-length. The gelation and retrogradation behaviour of the amylopectins, examined using measurements of shear modulus and differential scanning calorimetry, together with studies of the structure of the associated regions in amylopectin gels, indicated that association of the chains in cereal amylopectin gels occurred over shorter segment lengths. The cereal amylopectins have a reduced rate of retrogradation which is linked to their shorter average chain-lengths.

INTRODUCTION

The aggregation, from aqueous solutions and dispersions, of the starch component amylopectin is important in the food industry as it can lead to time-dependent changes in the texture and digestibility of food. Amylopectins from various botanical sources differ in their fine structure[1,2], and it is of interest to examine how the structure affects the aggregation behaviour.

Amylopectin is a highly branched polymer consisting of relatively short (1→4)-α-D-glucan chains which are joined together through α-(1→6) branch points. The chains have been classified in several ways[3,4]. In early studies of the fine structure of amylopectin, the chains were classified according to their linkage to the rest of the molecule. Thus, A chains are linked via the potential reducing end-groups, B chains are linked in this way and also carry one or more A chains, and the C chains carry the reducing end group. Amylopectins from various botanical sources have approximately equal numbers of A and B chains[4]. If amylopectin is debranched using an enzyme such as isoamylase, the distribution of chain lengths can be examined by size-exclusion chromatography. Many amylopectins give a bimodal distribution of chain lengths with d.p. ~40–60 and 15–20, the shorter chains being the most abundant. The ratio by weight of short to long chains varies between 3:1 and 12:1 depending on the source of the amylopectin[1]. Some cereal

0008-6215/90/$ 03.50

amylopectins, *e.g.*, wheat and barley, have a trimodal distribution of chain lengths[1,5]. Improved chromatographic procedures have shown[2] that tri- and polymodal distributions are more common than supposed previously. Thus, the major variations in the fine structure of amylopectin are the type of distribution of chain lengths, the ratio of short to long chains, and the chain length. In some amylopectins, *e.g.*, potato, ~1 residue in 300 is phosphorylated. An important feature of the structure of amylopectin is the size of the molecule, and mol. wts. in the region of 10^7–10^8 have been reported. The amylopectin molecule contains many short branches and, whereas enzymic methods have been useful in defining the types of chains that are present, only limited information on the overall structure has been obtained.

There is a correlation between the crystalline structure of the starch granule and the fine structure of amylopectin[1]. Three types (A–C) of wide-angle X-ray powder diffraction pattern have been obtained from native starch granules. Generally, pattern A is given by cereal starches, pattern B by tuber and fruit starches, and pattern C by legume starches. The average chain-length of amylopectins extracted from A-type starches was shorter than those extracted from B-type starches, and those of amylopectins extracted from C-type starches were intermediate. The crystallization behaviour of malto-oligosaccharides is dependent also on chain length[6,7]. Chains of d.p. 10–12 crystallized in the A-form, whereas higher oligomers crystallized in the B-form.

The aggregation and gelation behaviour of a waxy-maize amylopectin has been examined[8]. Aqueous solutions of >10% amylopectin concentration gelled slowly on quenching to 1°. The turbid elastic gel gave a wide-angle X-ray diffraction pattern of the B-type, and gel structure and crystallinity could be abolished by heating to 100°. Gelation occurred as a result of the separation of a partially crystalline phase. Structural studies[8] suggested that chain–chain association occurred over 15 or so residues. The association of amylopectin chains in partially crystalline domains was substantial and, therefore, a major fraction of the short constituent amylopectin chains was involved.

In extending this study, we have examined the relationship between the fine structure of amylopectins from various botanical sources and their gelation behaviour.

EXPERIMENTAL

Materials. — Starches from pea, potato, wheat, maize, canna, and barley were isolated by an aqueous extraction procedure[9]. The amylopectins were obtained after precipitation of the amylose and intermediate material with thymol[10]. Pullulanase (EC 3.2.1.4.1) from *Enterobacter aerogenes* and isoamylase (EC 3.2.1.6.8.1) were obtained from Hayashibara (Okayama, Japan), and sweet-potato beta-amylase (EC 3.2.1.2) from Koch–Light.

Physical methods. — The specific viscosities of aqueous solutions (0.25–3 mg/g)

of amylopectin were determined at 25° using a Ubbelohde suspended-level viscometer. The intrinsic viscosity was derived after extrapolation to zero concentration. At low concentrations of salt, the intrinsic viscosities of potato and canna amylopectins were dependent on ionic strength, indicating that they were polyelectrolytes. At salt concentrations >0.05M NaCl, the intrinsic viscosity of these amylopectins became independent of ionic strength. Measurements of shear modulus were performed[11] using a Rank Brothers "Pulse Shearometer". Differential scanning calorimetry (d.s.c.) was performed using a Perkin–Elmer DSC-2B instrument; details of the calibration and operation of the instrument have been reported[12].

Structural methods. — Enzyme hydrolysis, using beta-amylase, pullulanase, and isoamylase, was performed as described[8]. Heterogeneous acid hydrolysis was performed as described[8]. Debranched amylopectin and lintnerised residues were fractionated using a Toya-Soda TSK 3000 PWXL column (30 × 0.78 cm i.d.), preceded by a PWH guard column. The column was eluted with water at 0.5 mL/min. The elution of carbohydrate was monitored by using a refractive index detector. The column was calibrated with fractionated malto-oligosaccharides of known d.p.

RESULTS AND DISCUSSION

Structural studies. — The amylopectins were extracted from pea, canna, potato, maize, barley, and wheat starches. The specific viscosities of solutions of the purified amylopectins in 0.05M NaCl, determined with a capillary viscometer, ranged from 90–93 $mL.g^{-1}$, indicating that the sizes of the molecules were similar (Table I). The values are comparable to data on other amylopectins[3] and indicate that the amylopectins were of high molecular weight, *i.e.*, $>10^6$.

The chain profile of the amylopectins was examined by size-exclusion chromatography after debranching with isoamylase. No material was eluted at the void volume, indicating the absence of high-molecular-weight material, and the

TABLE I

PHYSICAL AND CHEMICAL CHARACTERISTICS OF AMYLOPECTINS[a]

Amylopectin	*Intrinsic viscosity* ($mL.g^{-1}$)	*D.p.*[b]		*Weight ratio F1:F2*	*Number ratio F1:F2*
		F1	*F2*		
Pea	93	50	16	1:3.9	1:12
Canna	92	55	16	1:2.4	1:8.0
Potato	93	60	18	1:2.1	1:7.2
Maize	90	45	15	1:3.5	1:10.6
Barley	92	50	14	1:4.8	1:17.3
Wheat	90	49	13	1:4.8	1:17.4

[a]Each value is the average of three determinations. [b]Variation ±1%; recorded to the nearest integer.

separations are shown in Fig. 1. Most of the elution profiles revealed two main components with average d.p. in the ranges 45–60 and 13–18. All distributions showed overlapping peaks and, following the previous convention[1], the profiles were divided into two sub-fractions F1 and F2 at the minimum of the elution curve between F1 and F2 (see Fig. 1), and the peak areas were measured (Table I). Where the maximum between F1 and F2 was not pronounced, *e.g.*, pea amylopectin, the inflexion point between F1 and F2 was used. For barley and wheat amylopectins, the F2 peak had a shoulder, indicating a trimodal distribution of chains in agreement with previous findings[1,5]. The d.p. of the material at the peak maxima of F1 and F2 was measured. Where the F1 fraction did not show a clear maximum (wheat, pea, and maize), the average d.p. was assigned to the mid-point of the plateau (Table I). The distribution profile was also calculated on a numerical basis, and the molar ratio of fractions F1 and F2 was determined as suggested by Palmer *et al.*[13]. Analysis of distribution profiles from three separate extracts of pea and maize amylopectin did not show an increased variation in the d.p. of F1 and F2. The amylopectins extracted from the cereal starch granules had a shorter and more abundant F2 fraction than the potato and canna amylopectins. These results suggest that amylopectins extracted from A-type starches have a shorter average chain-length. Comparison of the present results with published work[1] shows that, for the same botanical source, variations are observed in both the d.p. of F1 and F2 and the ratio of F1:F2. Whereas some of the variation may be due to the different experimental methods used, it is probable that varietal and climatic factors have an important influence.

Gelation studies. — Concentrated solutions (25% w/w in 0.05M NaCl) of the various amylopectins were gelled by quenching to 1°. After a few days, the solutions became turbid and, eventually, opaque elastic gels were formed. The shear modulus of the gels was monitored and, after 30 days, it had approached a limiting plateau value (Table II). The shear modulus varied from 0.8×10^4 N.m^{-2} for a wheat amylopectin gel to 3.6×10^4 N.m^{-2} for a pea amylopectin gel. The aggregation and crystallization of amylopectin, which can be reversed[8] by heating to 100°, was followed by d.s.c. An endothermic transition, corresponding to gel melting, was observed at 50–60°. The characteristics of the endotherm ΔH and the temperature at which the peak maximum occurred are shown in Table II. The ΔH for the transition varied between 5.3 mJ/mg of polysaccharide for the wheat amylopectin to 7.4 mJ/mg of polysaccharide for the canna amylopectin. There was no simple relationship between ΔH and shear modulus and no obvious correlation between shear modulus and chain profile, indicating that the overall structure, *e.g.*, heterogeneity of branching of the different amylopectins, may be important. The peak maximum temperature of the endothermic transition varied between 53° and 60°. The transition for wheat amylopectin occurred at 53 ±0.5°; for maize and barley amylopectin, at 55 ±0.5°; for pea amylopectin, at 57 ±0.5°; and, for potato and canna amylopectins, at 58 ±0.5° and 60 ±0.5°, respectively. The temperature of dissolution of the crystalline domains of the amylopectin gel depends on their

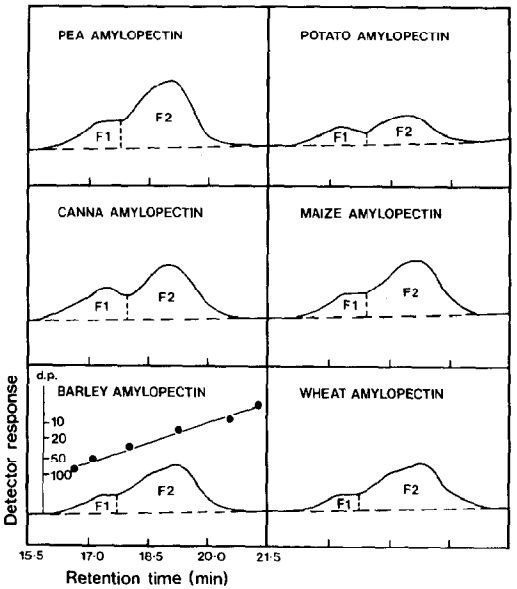

Fig. 1. Distributions of chain lengths for amylopectins.

perfection and size as well as the length of the chain involved in the association. The transition temperature for the cereal amylopectins, which have the shorter average chain-length, occurs at the lower temperature.

In order to obtain further information on the length of chain involved in the associated regions, the gels were subjected to heterogeneous acid hydrolysis. The technique has been used to determine the structure of polymer segments which form the crystalline domains of starch granules[14], amylose-V complexes[15], retrograded amylose, and amylopectin gels[8].

The residues obtained after hydrolysis for 30 days were examined by size-

TABLE II

PHYSICAL AND STRUCTURAL CHARACTERISTICS OF AMYLOPECTIN GELS[a]

Amylopectin	*Shear modulus* ($N.m^{-2}$)	*Transition temp.*[b] (°)	ΔH (*mJ/mg*)	*D.p.*[c]	
				Original residue	*Debranched residue*
Pea	3.6×10^4	57	7.2	64	18
Canna	2.4×10^4	60	7.4	47	19
Potato	1.4×10^4	58	6.2	55	19
Maize	2.4×10^4	55	6.6	64	16
Barley	1.9×10^4	55	7.1	64	16
Wheat	0.8×10^4	53	5.3	64	16

[a]Each result is the average of three determinations. [b]Variation ±0.5°. [c]Variation ±5%.

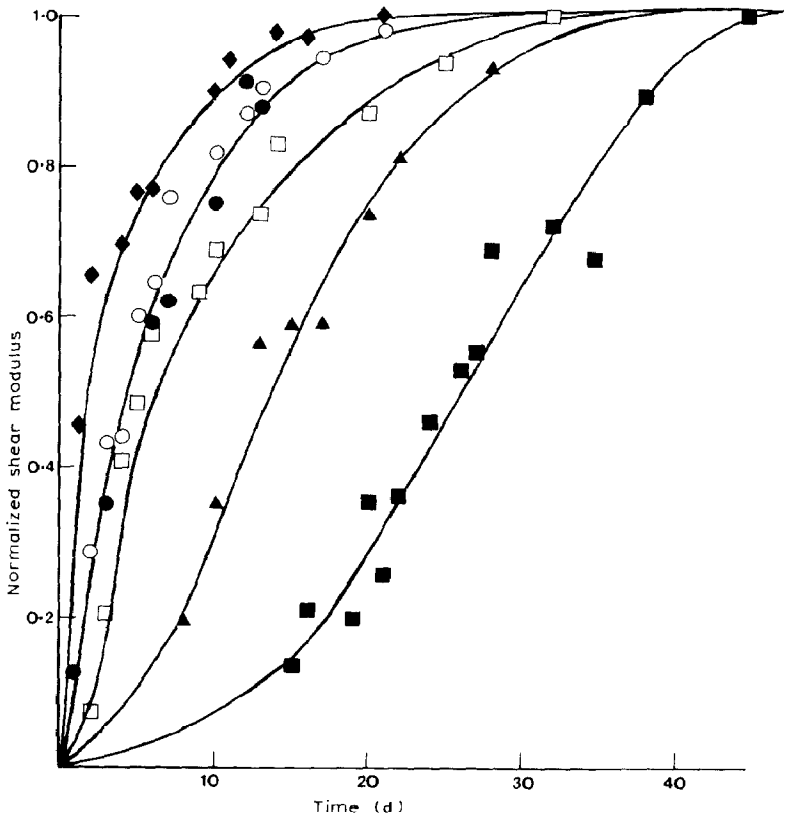

Fig. 2. Graph of shear modulus, normalised relative to its value after 30 days, *versus* time for pea (◆), potato (●), canna (○), wheat (■), maize (□), and barley (▲) amylopectins.

exclusion chromatography both before and after treatment with pullulanase (Table II). The time of elution of the residues was comparable to that of linear amylosic chains of d.p. 47–64. After debranching, a single peak was obtained; the debranched material was quantitatively hydrolysed by beta-amylase, indicating that it was linear. The maximum d.p. of the material in this peak (Table II) was 16 for the cereal amylopectins, 18 for the pea amylopectin, and 19 for the tuber amylopectins. This finding indicates that the association of chains in gels of cereal amylopectins occurs over fewer residues than in gels of pea, potato, and canna amylopectins.

Although there is no simple relationship between plateau shear modulus and molecular structure, it is useful to examine the rate of development of gel structure since the driving force for gelation and the kinetic factors limiting aggregation may be dependent on structure. Fig. 2 shows a plot of shear modulus, normalized relative to its value after 30 days, as a function of time. The rate of gelation decreased in the order pea, potato, canna, maize, barley, and wheat, and the cereal amylopectins had a delayed retrogradation. After 15 days, the modulus of the pea amylopectin gel had approached a limiting value, whereas that of the wheat

amylopectin gel was <20% of its final plateau value. Similar observations have been made on the retrogradation of amylopectin in starch gels[16].

The present results indicate that the length of the chains of amylopectin can have an important effect on the rate of aggregation. Other factors affect the extent of aggregation, and a more complete description of the variations in molecular structure of amylopectins from various botanical sources is required in order to establish further links between functionality and structure.

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