Carbohydrate Research, 110 (1982) 245–259
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

CAN CYCLODEXTRIN GLYCOSYLTRANSFERASE BE USEFUL FOR THE INVESTIGATION OF THE FINE STRUCTURE OF AMYLOPECTINS?: CHARACTERISATION OF HIGHLY BRANCHED CLUSTERS ISOLATED FROM DIGESTS WITH POTATO AND MAIZE STARCHES

HANS BENDER, RAINER SIEBERT, AND AGNES STADLER-SZÖKE*

Chemisches Laboratorium der Universität Freiburg i.Br., D-7800 Freiburg i.Br. (Federal Republic of Germany)

(Received March 29th, 1982; accepted for publication, May 25th, 1982)

ABSTRACT

Maize and potato amylopectin (57 and 64%, respectively) were recovered as non-cyclic products from 4-h digests of the starches with cyclodextrin glycosyltransferase {(1→4)-α-D-glucan:[(1→4)-α-D-glucopyranosyl]transferase (cyclising), EC 2.4.1.19} from *Klebsiella pneumoniae* M 5 al. Besides smaller saccharides, highly branched fragments of different sizes (average d.p. 40–140) were obtained by fractionation. The extents of beta-amylolysis varied between 24 and 37%, indicating that the clusters were not equally susceptible to attack by cyclodextrin glycosyltransferase. The fragments of potato amylopectin still contained larger amounts of material of high molecular weight. Accordingly, part of the longer B-chains of the basic structure were protected from the enzymic attack, presumably because of interchain branches. By debranching with pullulanase, it was evident that the beta-limit dextrins of the fragments of potato amylopectin were composed of longer B-chains (average chain-length 17.8) than those of maize amylopectin (average chain-length 14.1). The A/B-chain ratios, which were calculated from h.p.l.c. data for the debranched beta-limit dextrins, were 1.22 (maize) and 1.06 (potato). Some structural differences between potato and maize amylopectin are discussed.

INTRODUCTION

A description of the molecular structure of branched α-D-glucans requires knowledge of the molecular weight, the average chain-length ($\overline{c.l.}$), the relative lengths of the external and internal chains (extent of beta-amylolysis), and the ratio of A/B-chains[1]**. The investigation of the fine structure of these polysaccharides has been facilitated by the isolation of the bacterial debranching enzymes pullulanase[2]

*Present address: Chinoin Pharmaceutical and Chemical Works Ltd, 1325 Budapest, Hungary.
**An A-chain is connected to the remainder of the molecule only through its reducing chain-end. A B-chain is also joined in this way, but carries other A- and/or B-chains at one or more of its primary hydroxyl groups. A C-chain has one reducing terminus.

and isoamylase[3]. By the use of these enzymes, the $\overline{c.l.}$ of the linear chains and the A/B-chain ratios have been determined[4-7]. The chain profiles of most debranched amylopectins show a bimodal distribution, corresponding to long ($\overline{c.l.}$ 40–60) and short ($\overline{c.l.}$ 11–25) chains[6,8-12]. It is generally assumed that the chains are derived from a single, completely debranched amylopectin population. Based on these results, French[13,14] proposed the cluster model, whereby the shorter chains are arranged in tightly packed clusters throughout a basic structure composed of long chains[15,16]. This type of model not only explains the bimodal nature of chain lengths[17-20], but also accounts for the high crystallinity of native amylopectins and the high viscosity of amylopectin solutions[9,21,22]. Although some of the experimental results are in accord with a Meyer tree-like structure[8,23], the cluster model is generally accepted at present, whereas the elongated model proposed by Whelan[24,25] seems to be less probable.

The cyclodextrin glycosyltransferase {(1→4)-α-D-glucan:[(1→4)-α-D-glucopyranosyl]transferase (cyclising), EC 2.4.1.19, CGT} from *Klebsiella pneumoniae* M 5 al can catalyse simultaneously cyclisation (exo-attack) and chain-shortening (endo-attack) with both amylose and amylopectin. The effect of acceptors on these reactions has been studied with amylose[26,27]. Due to the initial chain-shortening reaction catalysed with amylopectin (which causes rapid liquefaction of amylopectin gels), fragments of markedly lower molecular weights are formed. As shown by the chain profile of the debranched fragments, the long B-chains seem to be the preferential substrate for the chain-shortening reaction[28,29]. In the course of prolonged incubation, CGT degrades the amylopectins into smaller saccharides, indicating that not only the long B-chains but also the shorter interbranch chains are attacked by the enzyme. Accordingly, the action of the CGT on branched α-D-glucans corresponds to a modified tier concept[18,30]. The complete degradation of the polysaccharide proceeds slowly, and depends on the lengths of the interbranch chains. The shorter the chains, the lower are their rates of degradation. Highly branched clusters, which are modified only in the region of A-chains by the cyclisation reaction, should be obtainable from short-term digests. We now describe the isolation and characteristics of some branched fragments from potato and maize starch.

EXPERIMENTAL

Materials. — CGT was isolated from the culture filtrate, and pullulanase [pullulan:(1→6)-glucanohydrolase, EC 3.2.1.41] from the cells, of continuously grown *Klebsiella pneumoniae* M 5 al, with purification as described[2,28,31-33]. The specific activity of the electrophoretically homogeneous CGT with (1→4)-α-D-glucopyranosyl chains ($\overline{c.l.}$ 20) was 2.6×10^5U/g of protein[34]. Amylose impurities of the pullulanase were removed by affinity chromatography on Sepharose–cyclohexaamylose[35]. Isoamylase [glycogen:(1→6)-glucanohydrolase, EC 3.2.1.68] was isolated from the culture filtrate of *Pseudomonas amyloderamosa* ATCC 21 262, and purified as described[26,36]. Beta-amylase [(1→4)-α-D-glucan:maltohydrolase, EC

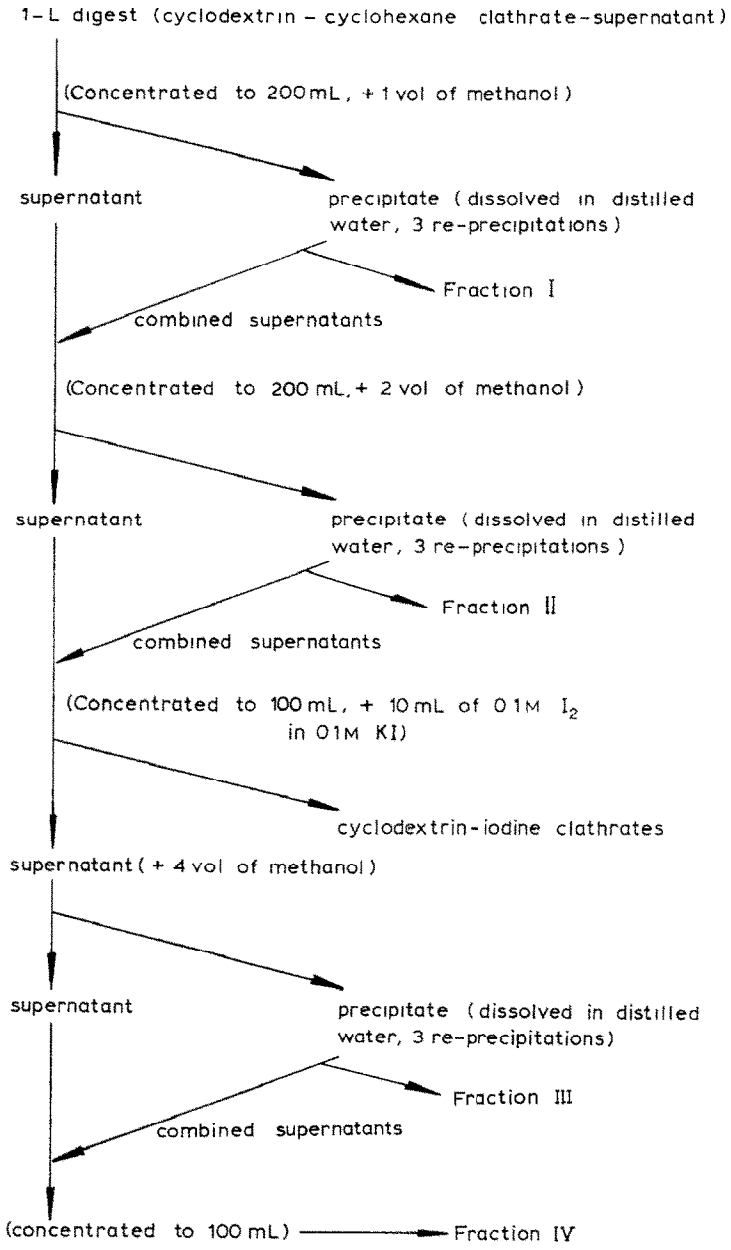
3.2.1.2, sweet potato, 500 U/mg of protein, thrice crystallised] was purchased from Serva (Heidelberg, Germany), and glucoamylase [(1→4),(1→6)-α-D-glucan:glucohydrolase, EC 3.2.1.3, *Aspergillus niger*, 14 U/mg of protein] and the glucose test-kit GOD-Perid were obtained from Boehringer (Mannheim, Germany). Potato and maize starches were a gift from Henkel & Cie (Düsseldorf, Germany). Amylopectin from potato starch (mol.wt. 2–4 × 10^6, 95% pure) was purchased from Serva (Heidelberg, Germany), and waxy maize starch from Koch–Light Laboratories Ltd. (Colnbrook, Great Britain). All other substances were commercial materials of the highest purity available.

Analytical methods. — Total carbohydrates were determined with anthrone[37,38], and reducing aldehyde groups with the Nelson reagent[39]. $\overline{C.l.}$ and d.p. values were calculated[40] as the ratios of total carbohydrate to the reducing carbohydrate, both in glucose equivalents. D-Glucose was determined with D-glucose oxidase[41], and protein by the biuret method[42]. The iodine complexes of the compounds were prepared by mixing water (9.8 mL) and aqueous 3% carbohydrate (0.2 mL) with 50mM iodine in 50mM potassium iodide (0.5 mL).

For debranching, aqueous 3% substrate solutions (10 mL) in 10mM acetate buffer (pH 5.6 or 3.6) were incubated either with pullulanase (2 U/mL) or with isoamylase (0.3 U/mL) at 30° for 72 h. For beta-amylolysis, 1% substrate solutions in 20mM acetate buffer (pH 4.8, 500 mL) were incubated with 6,000 U of beta-amylase at 30° for 72 h. The digests were concentrated *in vacuo*, and the beta-limit dextrins were precipitated with methanol (1–4 vol.), and purified by repeated re-precipitation.

H.p.l.c. was performed on Waters μBondapak-NH_2 columns (3.9 × 300 mm), using acetonitrile–water (65:35) at 1.5 mL/min (1,200 p.s.i., 25°), with refractometric detection. 2–10% Solutions of carbohydrate (20 μL) were injected. The G_2–G_3-stubs were determined by h.p.l.c. of the beta-limit dextrins of the fractions debranched by pullulanase. The elution peaks were calibrated by using maltose and maltotriose. As the total chain-profile could not be obtained by this method, the A/B-chain ratios were calculated from the total carbohydrate contents of the samples. The $\overline{c.l.}$ of the B-chains was calculated from the differences of the total reducing capacities of the samples and the reducing capacities of the G_2–G_3-stubs.

Gel chromatography of the branched fragments was carried out with columns (2 × 87 cm) of Sepharose 2B or Sepharose CL-6B (Pharmacia). A solution of carbohydrate (6 mg) in dimethyl sulfoxide (0.3 mL) was diluted to 6 mL with water, added to the column[43], and eluted (descending) with distilled water (20°). Fractions (4.5 mL) were collected at 11 mL/h, and the carbohydrate content was determined with anthrone. The chain profiles of the debranched fragments were obtained by chromatography on Sephadex G-50 (fine; Pharmacia). A solution of carbohydrate (6 mg) in distilled water (5 mL) was added to the column (2 × 90 cm), and eluted (descending) with 10mM sodium/potassium phosphate buffer[44] (pH 7.0). Fractions (4.5 mL) were collected at 10.4 mL/h, and the carbohydrate content was determined with anthrone.



Scheme 1. Fractionation of non-cyclic compounds from the digests with maize and potato starch.

Isolation of branched fragments on a preparative scale. — The digests contained potato and maize starches, their amylopectins[45], potato amylopectin (Serva), and waxy maize starch (Koch–Light). Satisfactory results for the isolation of non-cyclic products were obtained with 4-h digests of 10% substrate solutions. In order to avoid any reverse reactions of the initially formed cyclohexaamylose, cyclohexane was added, since cyclohexaamylose clathrates are resistant[46] to CGT. Similar results were obtained with purified amylopectins and the corresponding native starches, indicating that the part of the amylose that was not retrograded by the initial chain-shortening reaction[26,27] was converted into cyclodextrins. In contrast, 57% of the maize amylopectin and 64% of the potato amylopectin were recovered from the starch digests as non-cyclic compounds. Accordingly, carbohydrate (100 g) was dissolved in 5mM $CaCl_2$ (1 L) by boiling (the maize starch was heated to 134° for 2 h). After cooling to 35°, the pH was adjusted to 6.5, cyclohexane (50 mL) was added, and the transfer reactions were started by the addition of CGT (30 mg). The digests were stirred at 35° for 4 h, and the pH was then adjusted to 3 to inactivate the enzyme. After storage at 4° overnight, the cyclodextrin–cyclohexane clathrates were removed by centrifugation, together with some insoluble starch material. The total amounts of cyclic products were determined by h.p.l.c. The fractionation of the non-cyclic compounds is summarised in Scheme 1. Solutions of the final precipitates in distilled water were freeze-dried. Samples of the fractions were digested with glucoamylase. The carbohydrates were completely degraded into D-glucose, indicating that they contained no cyclic compounds.

RESULTS AND DISCUSSION

Mode of action of CGT on amylopectins. — The time-dependent degradation of amylopectin into fragments of lower molecular weights by CGT is shown in Fig. 1.

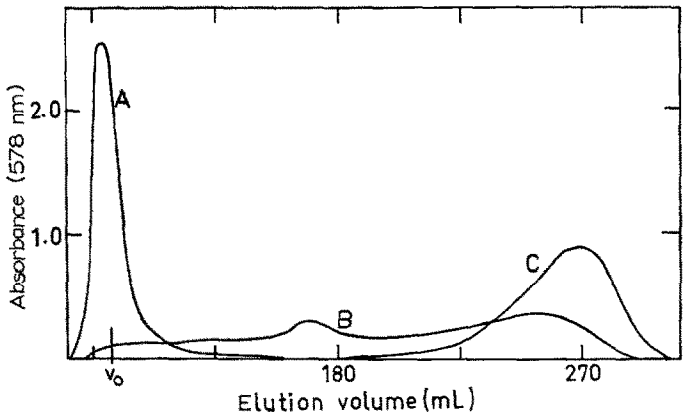


Fig. 1. Elution profiles from Sepharose 2B of waxy maize starch (*A*), and of waxy maize starch (3%) incubated with CGT (14 mg/L) for 10 (*B*) and 80 min (*C*) at 30° (for the conditions of gel filtration, see Experimental).

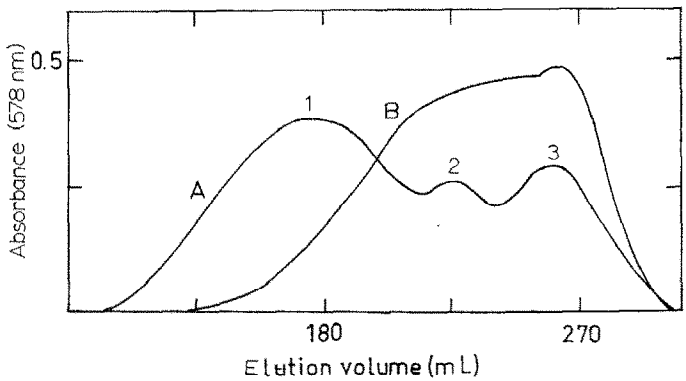


Fig. 2. Sephadex G-50 fractionation of *A*, the pullulanase-debranched beta-limit dextrin of potato amylopectin; and *B*, the pullulanase-debranched fragments obtained after incubation of the beta-limit dextrin (3%) with CGT (14 mg/L) at 30° for 40 min (for the preparation of the beta-limit dextrin, debranching, and chromatographic methods, see Experimental).

The exclusion limit of Sepharose 2B is too low to provide a true representation of the molecular weight distribution of the native amylopectin. The majority of carbohydrate was eluted in the void volume of the column (Fig. 1A). During the incubation, smaller fragments were formed, which were included into the gel (Fig. 1B,C). Due to the degradation of the A-chains, the population of longer B-chains can be seen best by debranching the beta-limit dextrins. Incubation of the beta-limit dextrins with pullulanase followed by gel filtration of the digests yielded a trimodal distribution of carbohydrate[9,12] (Fig. 2A) corresponding to long B-chains (peak 1), to B-chains of intermediate lengths (peak 2), and to shorter B-chains including the G_2–G_3-stubs (peak 3). The amount of long B-chains markedly diminished after incubation of the CGT with the branched α-D-glucan for 40 min (Fig. 2B). Accordingly, the degradation of the long B-chains is responsible for the formation of the smaller fragments.

Characterisation of the non-cyclic degradation products isolated from digests of maize and potato amylopectin with CGT. — As shown by chromatography of the amylopectin digests, the non-cyclic compounds were very polydisperse (Fig. 1). The range of their molecular sizes was decreased, therefore, by fractionation from methanol–water mixtures. The yields of the fractions, and some of their characteristics, are summarised in Table I. The extent of beta-amylolysis varied between 24 and 37%, indicating that the compounds were not equally susceptible to attack by CGT.

The beta-limit dextrins of the fractions were subjected to gel filtration on Sepharose CL-6B (Fig. 3A,B). The Pβ-fragments contained material of high molecular weight, which was eluted in or near the void volume of the column. These compounds (PIβ, 71%; PIIβ, 57%; and PIIIβ, 24% of the total carbohydrate) apparently represented molecules modified only in the exterior regions. As shown by prolonged incubation, some material of high molecular weight could be isolated from 15-h digests, indicating that at least some of the long B-chains were resistant to attack by CGT. Presumably they contain interchain branches, which prevent rapid degradation.

TABLE I

YIELDS AND SOME CHARACTERISTICS OF NON-CYCLIC COMPOUNDS OBTAINED BY FRACTIONATION FROM METHANOL–WATER MIXTURES OF THE DIGESTS WITH MAIZE (M) AND POTATO (P) STARCH[a]

Substrate	*Fraction*[b]	*Amylopectin* (%)	*D.p.*[c]	*Beta-amylolysis*[d] (%)
M	I	19	1,500	24
	II	12	250	36
	III	13	98	29
	IV	13	20	n.d.
P	I	26	717	37
	II	10	220	24
	III	9	83	24
	IV	19	20	n.d.

[a]A 10% solution (1 L) of starch in 5mM $CaCl_2$ (pH 6.5) was incubated with CGT (30 mg) at 35° for 4 h in the presence of cyclohexane (50 mL). The insoluble cyclodextrin–cyclohexane clathrates were removed by centrifugation; 46.6% (M) and 46.1% (P), respectively, of the starches were converted into cyclodextrins (80% cyclohexaamylose); 12.5% (M) and 5.9% (P) of the starches were not dissolved by heating, or became insoluble by retrogradation. [b]For the fractionation of the non-cyclic compounds, see Experimental. [c]Calculated from the ratios of total carbohydrate/reducing capacity[40]. [d]For the conditions of beta-amylolysis, see Experimental.

The population of the long B-chains of maize amylopectin, however, must be fully susceptible to attack by CGT.

The d.p. values calculated from the reducing power[40] were 492 for MIβ and 454 for PIβ (Table II). The former value was inconsistent with the partition coefficient on the Sepharose gel, and inconsistent with the d.p. calculated for MI (24% of beta-amylolysis). The d.p. of this fraction clearly could not be determined exactly by the method of Gunja-Smith *et al.*[40]. The d.p. values calculated for the P-fractions, however, seemed to be more realistic. Assuming that the reducing capacities of the materials of high molecular weight are near zero, then d.p. values of 130 [PIβ, average mol. wt. ($\overline{M}_n$) 21,000], 78 [PIIβ, 12,600], and 48 [PIIIβ, 7,800] could be calculated for the compounds included into the gel. Provided that the Mβ fractions behaved similarly during gel filtration, MIβ and the majority of MIIβ must be somewhat larger, and MIIIβ somewhat smaller, in size than the corresponding Pβ fractions.

Both dextran T40 ($\overline{M}_w$ 39,500, $\overline{M}_n$ 29,500; Pharmacia) and amylose ($\overline{M}_n$ 136,000 $\pm 5\%$[26], Serva) were subjected to chromatography on Sepharose CL-6B (Fig. 3C). The amylose was very polydisperse. From the elution pattern of the dextran, it was evident that the $\overline{M}_n$ values of MIβ and the minor part of PIβ must be $<29{,}500$ but $>15{,}000$, since the fragments were not dialysable (Visking tubing, exclusion limit 15,000 daltons). In contrast, 40% of MIIβ was dialysable, and MIIIβ was completely dialysable.

Each of the fractions was a very poor substrate for the cyclisation reaction. The rate of cyclisation for amylopectin is[26,34] 160 U/mg of CGT. For MI and PI,

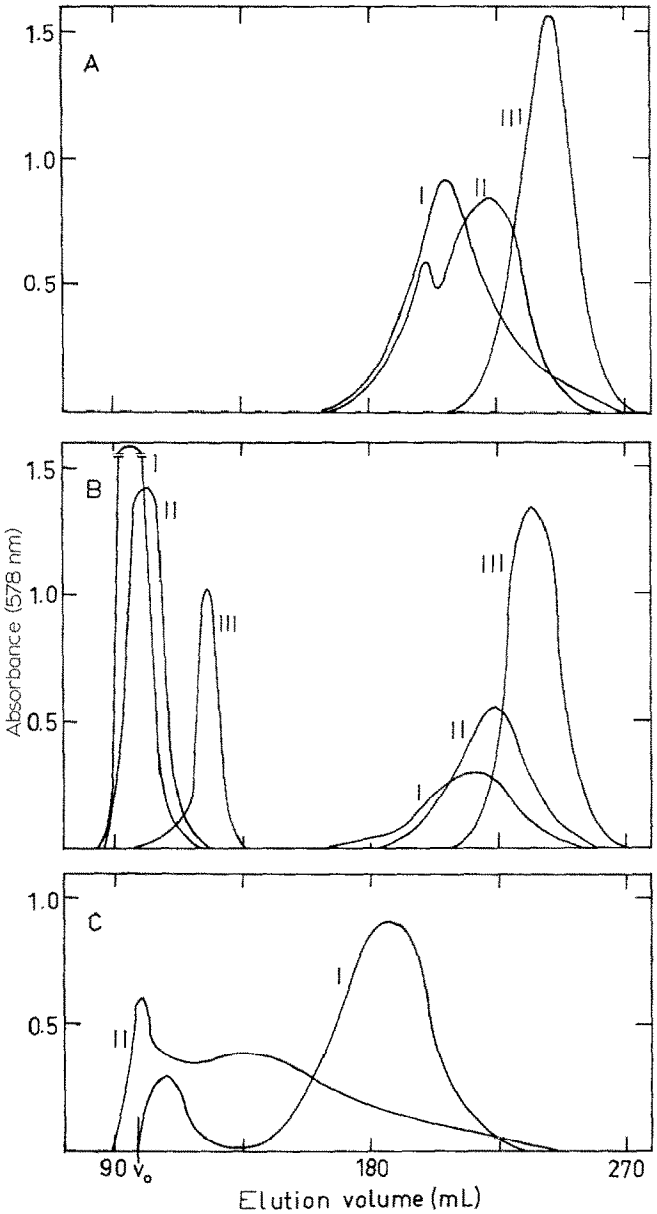


Fig. 3. Elution profiles from Sepharose CL-6B of the fractions MIβ–MIIIβ (*A*, I–III) and PIβ–PIIIβ (*B*, I–III; parts of the fractions were eluted in or near the void volume of the column: peaks I–III on the left), and of dextran T40 (*C*, I) and amylose (*C*, II) (for fractionation, preparation of the beta-limit dextrins, and the chromatographic methods, see Experimental).

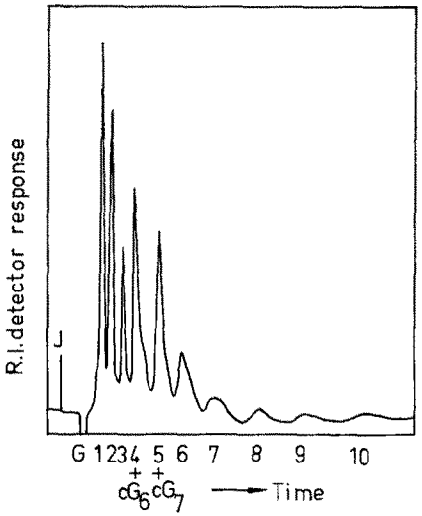


Fig. 4. H.p.l.c. (25°) of the products obtained by incubation of MI (5%) with CGT (30 mg/L) at 30° for 72 h; the digest injected (20 μL) contained 0.7 mg of carbohydrate; the d.p. value[40] was 5.8. For the conditions of h.p.l.c., see Experimental).

TABLE II

ANALYSIS OF THE BETA-LIMIT DEXTRINS OF THE FRACTIONS FROM MAIZE (M) AND POTATO (P) AMYLOPECTIN[a]

Fraction	*D.p.*[b]	*Iodine colour*	*Debranching with pullulanase*[c]			
			C.l. of B-chains	*Branching (%)*[d]	*G_2–G_3-stubs (%)*	*A/B-chain ratios*
MIβ	470	Red	13.6	14	18.3	1.23
MIIβ	159	*Red*-brown	13.7	13	17	1.16
MIIIβ	70	Red-*brown*	15	12	17.4	1.27
PIβ	454 (130)	Red-*violet*	20	9.2	11.5	1.02
PIIβ	181 (78)	*Red*-violet	17	11.3	15.1	1.15
PIIIβ	63 (48)	Brown-*red*	16.4	11.1	13.8	1

[a]For the preparation of the beta-limit dextrins, see Experimental. [b]Calculated from the ratios of total carbohydrate/reducing capacity[40]. D.p. values were calculated for the parts of the carbohydrate that were included into the gel (chromatography on Sepharose Cl-6B). [c]For the conditions of debranching, see Experimental. The G_2–G_3-stubs were determined by h.p.l.c. The A/B-chain ratios were calculated from the total carbohydrate content of the debranched samples. The c.l. values for B-chains were calculated from the differences of the total reducing capacities and the reducing capacities of the G_2–G_3-stubs. [d]Percentage of reducing chain-ends of total carbohydrate (both in glucose equivalents) liberated by debranching.

the values were 24 and 32 U/mg of protein, respectively. The beta-limit dextrins were not substrates for cyclisation. MI and PI and their beta-limit dextrins were degraded into smaller branched, and, by disproportionation, linear oligosaccharides during prolonged incubation with CGT. Unbranched cyclodextrins could be detected only in the digests of MI and PI (Fig. 4). Thus, the enzyme could not form cyclic products from the highly branched regions of the molecules, but could degrade them by endo attack [hydrolytic activity (?) of CGT[47,48]].

Chain composition of the fragments; A/B-chain ratios. — Evidently, MI–MIII and the smaller compounds of the PI–PIII fragments represented populations of tightly packed clusters. It was to be expected that the total Pβ fraction still contained long B-chains of the basic structure. Debranching of the Pβ fragments with pullulanase yielded linear chains, which were markedly longer than those in the Mβ fractions (Table II). PIβ contained a high percentage of retrograded chains (molar ratio of long and short chains, 1 : 1.5). Only part of the long chains could originate from the basic structure, whereas the remainder must have been derived from the clusters.

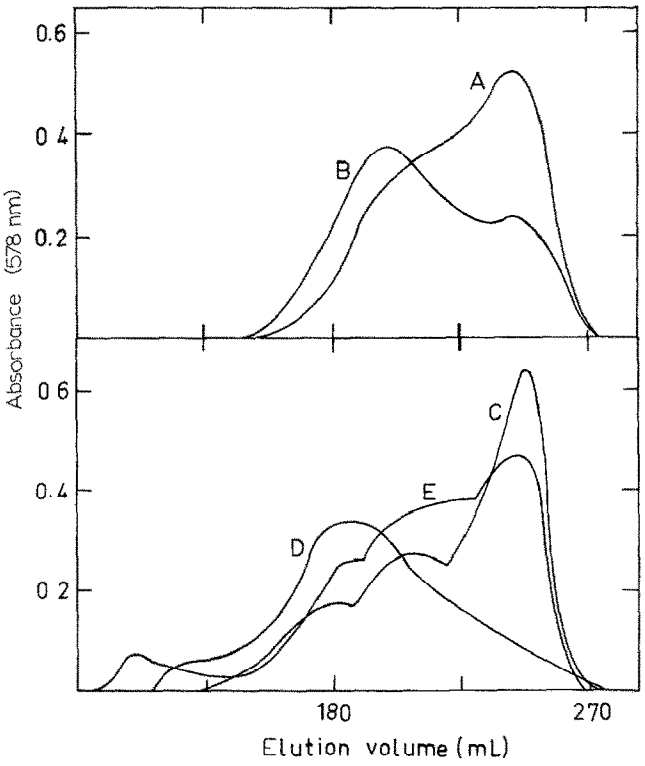


Fig. 5. Sephadex G-50 fractionation of the pullulanase-debranched beta-limit dextrins of the fragments isolated from digests with maize and potato starch: *A*, MIβ (4°-supernatant); *B*, MIβ (retrograded material, 12% of total carbohydrate); *C*, PIβ (4°-supernatant); *D*, PIβ (retrograded material, 52% of total carbohydrate); *E*, PIIβ (total carbohydrate). For the preparation of the beta-limit dextrins, debranching, and chromatographic methods, see Experimental.

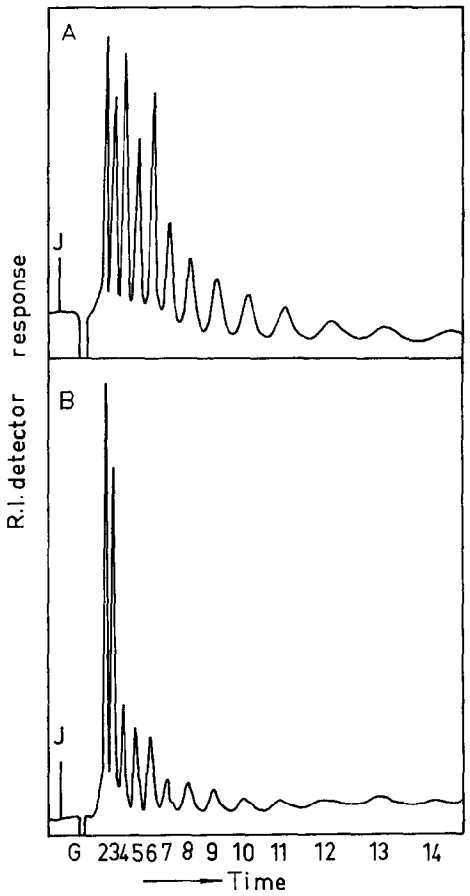


Fig. 6. H.p.l.c. (25°) of the 4°-supernatants of *A*, pullulanase/isoamylase-debranched MI; and *B*, pullulanase-debranched MIβ. The amount of carbohydrate injected (20 μL) was 2 mg for MI and 0.9 mg for MIβ. For the preparation of the beta-limit dextrins, debranching, and the conditions of h.p.l.c., see Experimental.

Of the G_2–G_3-stubs, ~50% should be localised on the longer chains, as indicated by comparison of pullulanase and isoamylase digests (see below).

The h.p.l.c. method used in the present work was unsuited for the analysis of the total chain-profiles of the fragments (the reverse-phase procedure[49] could not be reproduced). Rough chain-profiles were obtained by gel filtration[11,42,44] of the pullulanase-debranched, beta-limit dextrins on Sephadex G50 (fine). The distribution pattern confirmed that MIβ (4° supernatant) contained more shorter chains than the Pβ fractions. The gel filtration yielded a slightly bimodal distribution of carbohydrate (Fig. 5A). Even with the retrograded chains (4°-insoluble material, 12% of total carbohydrate), long B-chains could not be detected (Fig. 5B). Similar chain-profiles were obtained with MIIβ and MIIIβ. The PIβ (4° supernatant) yielded a trimodal distribution of carbohydrate (Fig. 5C). Most of the longer chains were re-

covered with the 4°-insoluble material (52% of total carbohydrate, Fig. 5D). PIIβ (PIIIβ) contained small amounts (4.5% of the total carbohydrate) of very long B-chains (Fig. 5E). Apparently, most of the long B-chains had been shortened to c.l. 30–35 by the action of CGT and beta-amylase, and therefore must have carried the first branch remote from the non-reducing chain-ends. In contrast, part of the long B-chains of PIIβ (PIIIβ, material of high molecular weight) must be protected from enzymic attack, presumably by carrying interchain branches or branched clusters near the non-reducing chain-ends. The amounts of chains retrograded at 4° were larger in the debranched fragments than in the corresponding debranched beta-limit dextrins, indicating that part of the retrograded longer chains was either A-chains, or B-chains shortened by beta-amylolysis. Larger amounts of retrograded chains were obtained with the pullulanase than with the isoamylase digests. Accordingly, part of the longer chains might carry G_2-stubs (and presumably equal amounts of G_3-stubs, originating from the action of CGT) near the non-reducing chain-ends, thereby preventing retrogradation. As shown by h.p.l.c., MI contained significant amounts of G_2–G_7-stubs (Fig. 6A; it is uncertain at present whether G_1-stubs were formed). Calculated from the different amounts of retrograded material obtained by debranching with pullulanase and isoamylase, all of the longer chains of, for example, MIβ (MIIβ) should be exterior B-chains.

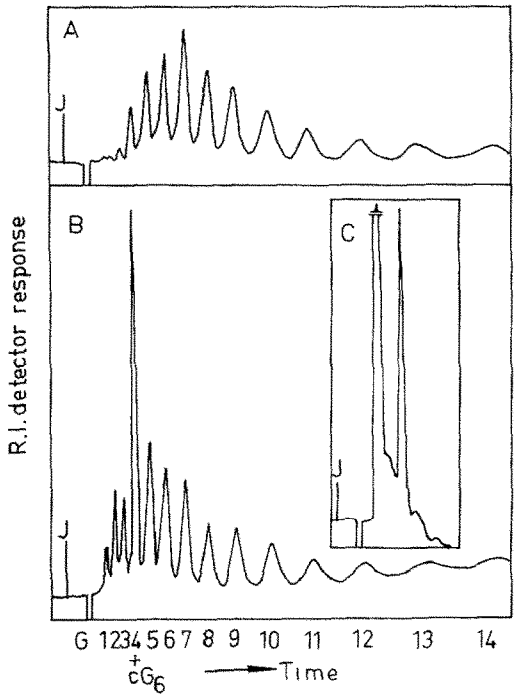


Fig. 7. H.p.l.c. of *A*, MIV; *B*, pullulanase-debranched MIV; and *C*, MIV degraded with glucoamylase. The digests injected (20 μL) contained 2 mg of carbohydrate. For conditions of h.p.l.c., see Experimental.

Determination of the A/B-chain ratios by the method of Marshall and Whelan[5,8] suffers from certain disadvantages. Therefore, the amounts of G_2–G_3-stubs were determined by h.p.l.c. of the pullulanase-debranched beta-limit dextrins (Table II). As an example, the chain profile (up to G_{14}) of debranched MIβ is shown in Fig. 6B.

Fractions MIV and PIV, as shown by debranching, were at least partly branched oligosaccharides. Most of the branches were shortened to G_2–G_3-stubs by the action of CGT. The small fragments appeared early in the digests, indicating that some exterior branched regions of the amylopectins were very susceptible to attack by CGT. Markedly larger amounts were obtained from the potato amylopectin than from the maize amylopectin (Table I). The A/B-chain ratios calculated were 0.98 for MIV (d.p. 14.5, 23.5% of G_2–G_3-stubs, percentage of branching 12.5) and 0.93 for PIV (d.p. 13.1, 15.6% of G_2–G_3-stubs, percentage of branching 5.6). MIV was subjected to h.p.l.c. (Fig. 7A) and shown to contain only traces of G_1–G_3. The larger saccharides had R_F values corresponding to malto-oligosaccharides up to G_{14}. H.p.l.c. of the pullulanase-debranched MIV revealed G_2–G_3-stubs (Fig. 7B). As shown by digestion with glucoamylase (Fig. 7C), the debranched MIV contained cyclohexaamylose which was not present in MIV. Accordingly, the cyclic compound must have been branched with a larger saccharide.

CONCLUSION

The ratios of chains $\overline{\text{c.l.}}$ 45/15 have been found to be 1:7.5 for wheat[12], and 1:6 for banana amylopectin[50]. Similar ratios were obtained for potato and cereal amylopectins[15,16]. Thus, the population of long B-chains amounts to ~25% of the total carbohydrate. Assuming that $\overline{M}_n$ of potato amylopectin (Serva) is 3×10^6, then 1 mol of amylopectin contains 114.3 mol of chains of $\overline{\text{c.l.}}$ 45. The $\overline{M}_n$ values of a single cluster and its beta-limit dextrin (56% beta-amylolysis) are, therefore, 26,250 (d.p. 162) and 11,560 (d.p. 71.3). At a molecular weight similar to that, the d.p. of the beta-limit dextrin (58% beta-amylolysis) of a cluster of maize amylopectin is 68. The experimental results show that maize and potato amylopectin contained at least three types of clusters, the beta-limit dextrins of which had d.p. values of 40–140. Calculated from the total yields of fractions, the average of the Pβ-clusters was d.p. 75, and that of the Mβ clusters was ~80 (the PIV and the MIV fractions were not considered in the calculation). MIIIβ and the bulk of PIIIβ were eluted from the Sepharose column in narrower ranges of molecular weight than was dextran T40. Thus, they must be clusters of very similar sizes.

There are marked differences in the structures of maize and potato amylopectin. The conditions of the digestion of each substrate with CGT were similar. Nevertheless, the fragments derived from the potato amylopectin contained marked amounts of longer B-chains; hence, the clusters are not so tightly packed. The structure seems to be looser, and the long B-chains of the basic structure carry interchain branches throughout their lengths. This supposition is strengthened by the isolation of larger amounts of small fragments (PIV) from short-term digests. In contrast, maize amylo-

pectin is composed of tightly packed clusters connected by more or less unbranched, long B-chains of the basic structure.

The $\overline{c.l.}$ values of the unit chain are 24 and 23 for potato and maize amylopectin, respectively (see ref. 51). As the B-chains are longer in the potato than in the maize amylopectin, the $\overline{c.l.}$ values of the A-chains must be larger in the maize amylopectin, which is the better substrate for the cyclisation reaction catalysed by CGT.

ACKNOWLEDGMENTS

This research was supported by grants from the Deutsche Forschungsgemeinschaft (Be 789/2, Be 789/3). A grant (to A.S.-S.) from the Deutsche Akademische Austauschdienst for a stay in Freiburg is gratefully acknowledged.

REFERENCES

1 S. PEAT, W. J. WHELAN, AND G. J. THOMAS, *J. Chem. Soc.*, (1952) 4546–4548.
2 H. BENDER AND K. WALLENFELS, *Biochem. Z.*, 334 (1961) 79–95.
3 T. HARADA, K. YOKOBAYASHI, AND A. MISAKI, *Appl. Microbiol.*, 16 (1968) 1439–1444.
4 E. Y. C. LEE, C. MERCIER, AND W. J. WHELAN, *Arch. Biochem. Biophys.*, 125 (1968) 1028–1030.
5 J. J. MARSHALL AND W. J. WHELAN, *Arch. Biochem. Biophys.*, 161 (1974) 234–238.
6 C. Y. LII AND D. R. LINEBACK, *Cereal Chem.*, 54 (1977) 138–149.
7 D. J. MANNERS AND N. K. MATHESON, *Carbohydr. Res.*, 90 (1981) 99–110.
8 Z. GUNJA-SMITH, J. J. MARSHALL, C. MERCIER, E. E. SMITH, AND W. J. WHELAN, *FEBS Lett.*, 12 (1970) 101–104.
9 H. AKAI, K. YOKOBAYASHI, A. MISAKI, AND T. HARADA, *Biochim. Biophys. Acta*, 252 (1971) 427–431.
10 E. Y. C. LEE AND W. J. WHELAN, *Arch. Biochem. Biophys.*, 116 (1966) 162–167.
11 L. F. HOOD, *Carbohydr. Res.*, 61 (1978) 53–66.
12 W. A. ATWELL, R. C. HOSENEY, AND D. R. LINEBACK, *Cereal Chem.*, 57 (1980) 12–16.
13 D. FRENCH, *Dempun Kagaku*, 19 (1972) 8–25.
14 M. YAMAGUCHI, K. KAINUMA, AND D. FRENCH, *J. Ultrastruct. Res.*, 69 (1979) 249–261.
15 J. P. ROBIN, C. MERCIER, F. DUPRAT, R. CHARBONNIERE, AND A. GUILBOT, *Staerke*, 27 (1975) 36–45.
16 J. P. ROBIN, C. MERCIER, R. CHARBONNIERE, AND A. GUILBOT, *Cereal Chem.*, 51 (1974) 389–406.
17 Y. IKAWA, D. V. GLOVER, Y. SUGIMOTO, AND H. FUWA, *Carbohydr. Res.*, 61 (1978) 211–216.
18 D. FRENCH, *M.T.P. Int. Rev. Sci., Org. Chem. Ser. One*, 5 (1975) 267–335.
19 M. ABDULLAH AND D. FRENCH, *Arch. Biochem. Biophys.*, 137 (1970) 483–493.
20 R. S. HALL AND D. J. MANNERS, *Carbohydr. Res.*, 83 (1980) 93–101.
21 K. UMEKI AND K. KAINUMA, *Carbohydr. Res.*, 96 (1981) 143–159.
22 D. BOROVSKY, E. E. SMITH, AND W. J. WHELAN, *Eur. J. Biochem.*, 59 (1975) 615–625.
23 K. H. MEYER, *Experientia*, 8 (1952) 405–420.
24 W. J. WHELAN, *Dempun Kagaku*, 23 (1976) 101–115.
25 D. BOROVSKY, E. E. SMITH, W. J. WHELAN, D. FRENCH, AND S. KIKUMOTO, *Arch. Biochem. Biophys.*, 198 (1979) 627–631.
26 H. BENDER, *Carbohydr. Res.*, 78 (1980) 147–162.
27 H. BENDER, *Carbohydr. Res.*, 101 (1982) 279–285.
28 H. BENDER, *Proc. Int. Symp. Cyclodextrins, Budapest, 1st*, Reidel, Dordrecht, 1982, 77–87.
29 R. SIEBERT, *Proc. Int. Symp. Cyclodextrins, Budapest, 1st*, Reidel, Dordrecht, 1982, 95–100.
30 J. LARNER, B. ILLINGWORTH, G. T. CORI, AND C. F. CORI, *J. Biol. Chem.*, 199 (1952) 641–651.
31 H. BENDER, *Arch. Microbiol.*, 111 (1977) 271–282.
32 H. BENDER, *Arch. Microbiol.*, 113 (1977) 49–56.
33 H. BENDER AND K. WALLENFELS, *Methods Enzymol.*, 8 (1966) 555–559.
34 H. BENDER, *Anal. Biochem.*, 114 (1981) 158–162.

35 B. S. ENEVOLDSEN, L. REIMANN, AND N. L. HANSEN, *FEBS Lett.*, 79 (1977) 121–124.
36 K. YOKOBAYASHI, A. MISAKI, AND T. HARADA, *Biochim. Biophys. Acta*, 212 (1970) 458–469.
37 R. G. SPIRO, *Methods Enzymol.*, 8 (1966) 3–36.
38 F. J. VILES AND L. SILVERMAN, *Anal. Chem.*, 21 (1949) 950–953.
39 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375–380.
40 Z. GUNJA-SMITH, J. J. MARSHALL, AND E. E. SMITH, *FEBS Lett.*, 13 (1971) 309–311.
41 J. B. LLOYD AND W. J. WHELAN, *Anal. Biochem.*, 30 (1969) 467–470.
42 G. BEISENHERZ, H. J. BOLTZE, T. BÜCHER, T. CZOK, K. H. GARBADE, E. MEYER-ARENDT, AND G. PFLEIDERER, *Z. Naturforsch, Teil B*, 8 (1953) 555–577.
43 D. PATON, *Staerke*, 31 (1979) 184–187.
44 L. F. HOOD AND C. MERCIER, *Carbohydr. Res.*, 61 (1978) 53–66.
45 G. K. ADKINS AND C. T. GREENWOOD, *Carbohydr. Res.*, 11 (1969) 217–224.
46 H. BENDER, *Carbohydr. Res.*, 78 (1980) 133–145.
47 S. KOBAYASHI, K. KAINUMA, AND S. SUZUKI, *Carbohydr. Res.*, 61 (1978) 229–238.
48 D. FRENCH, *Adv. Carbohydr. Chem.*, 12 (1957) 189–260.
49 G. B. WELLS AND R. L. LESTER, *Anal. Biochem.*, 97 (1979) 184–190.
50 K. KAYISU AND L. F. HOOD, *J. Food Sci.*, 46 (1981) 1894–1897.
51 C. T. GREENWOOD, in W. PIGMAN AND D. HORTON (Eds.), *The Carbohydrates, Chemistry and Biochemistry, Vol. 2B*, Academic Press, New York, 1970, pp. 471–513.