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PARTIAL CHARACTERISATION OF AMYLOPECTIN ALPHA-DEXTRINS

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ABSTRACT

Alpha-dextrins, obtained by fractional precipitation with methanol from hydrolysates of waxy-maize amylopectin after the action of *Bacillus subtilis* alpha-amylase, were analysed using sweet-potato beta-amylase and rabbit muscle phosphorylase. Those obtained after alpha-amylolysis for 60 min had similar phosphorolysis (39.5%) and beta-amylolysis limits (48.4%), and those obtained after 210 min had much lower limits. The limit values were higher for the smaller dextrins. The mean chain-length in the alpha-dextrins obtained after alpha-amylolysis for 60 and 210 min were 14.9 and 11.1, respectively, regardless of the d.p. The smaller chain-length in the latter alpha-dextrins was due to a shortening of the external chains. It is suggested (*a*) that the alpha-amylolysis involves two independent processes, namely, formation of maltohexaose from the external chains and of branched intermediate alpha-dextrins by fission of longer internal chains; and (*b*) that the phosphorolysis limit dextrins had three D-glucosyl residues on the external B-chains and four residues on the A-chains.

INTRODUCTION

The alpha-amylase of *Bacillus subtilis* has 9 sub-sites which bind the D-glucosyl residues of the substrate[1,2]. The sub-sites are distributed unevenly on both sides of the active site, so that maltohexaose and maltotriose are produced preferentially[1,3,4]. Small malto-oligosaccharides, such as maltohexaose, are degraded further very slowly, because they do not occupy all of the possible sub-sites. Therefore, effective binding of the substrate to the enzyme is decreased[1]. The ability of the enzyme to attack close to the (1→6) linkages in amylopectin and its β-limit dextrin is also restricted. The smaller branched α-limit dextrins produced by the saccharifying[5,6] and liquefying[7] enzymes of *B. subtilis* species have been studied in detail. The smallest, singly branched, α-limit dextrin is 6^2-α-maltosylmaltotriose[8,9].

Little work has been reported on the products obtained in the early stages of the alpha-amylolysis of amylopectin. These products have been regarded as a complex mixture of branched dextrins, obtained by a random action of the alpha-

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amylase[4], but dextrins (alpha-dextrins) of defined sizes[10] (and possibly of defined structure) appear to be present.

Beta-amylase from sweet potato hydrolyses maltose from the non-reducing ends of amylopectin, to give limit dextrins with two D-glucosyl residues on average next to the branches[11]. Rabbit muscle phosphorylase *a* produces D-glucose 1-phosphate from the non-reducing end units and leaves four D-glucosyl residues next to a branch point[12]. Maltotetraose and smaller malto-oligosaccharides are resistant to this phosphorylase[13].

The purpose of the study now reported was to characterise the intermediate alpha-dextrins obtained by the action of *B. subtilis* alpha-amylase on amylopectin and partly purified by methanol precipitation[14].

EXPERIMENTAL

Substrates and enzymes. — Waxy-maize starch granules (amylopectin, Sigma) were deproteinised and defatted as described[11]. The alpha-dextrins were those produced from waxy-maize starch by the action of alpha-amylase of *Bacillus subtilis* [(1→4)-α-D-glucan glucanohydrolase, EC 3.2.1.1; Koch–Light], and isolated[14] by fractional precipitation with methanol.

Beta-amylase from sweet potato [(1→4)-α-D-glucan maltohydrolase, EC 3.2.1.2; Sigma], purified[14] by ion-exchange chromatography[15], had an activity of 5000 U/mL.

The following enzymes, purchased from Boehringer–Mannheim, were used without further purification: phosphorylase *a* from rabbit muscle [(1→4)-α-D-glucan:orthophosphate α-D-glucosyltransferase, EC 2.4.1.1], phosphoglucomutase from rabbit muscle (α-D-glucose 1,6-bisphosphate:α-D-glucose 1-phosphate phosphotransferase, EC 2.7.5.1), α-D-glucosidase from yeast (α-D-glucoside glucohydrolase, EC 3.2.1.20), amyloglucosidase of *Aspergillus niger* [(1→4)-α-D-glucan glucohydrolase, EC 3.2.1.3], hexokinase from yeast (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1), D-glucose 6-phosphate dehydrogenase from yeast (D-glucose 6-phosphate:NADP oxidoreductase, EC 1.1.1.49), glycerol kinase of *Candida mycoderma* (ATP:glycerol 3-phosphotransferase, EC 2.7.1.30), and glycerol 3-phosphate dehydrogenase from rabbit muscle (sn-glycerol 3-phosphate:NAD 2-oxidoreductase, EC 1.1.1.8).

The alpha-dextrins were dissolved in water to give stock solutions of 5 mg/mL. A stock solution of amylopectin (2.5 mg/mL) was made by gelatinisation of the waxy-maize starch granules on a boiling water bath for 15 min. All stock solutions were prepared freshly.

Total carbohydrate analysis. — An aliquot (0.1 mL) of the stock solution was diluted with water (2.4 mL). To 0.1 mL of this solution were added 0.05M citrate buffer (0.2 mL, pH 4.6) and amyloglucosidase (20 μL); the mixture was incubated at 55–60° for 15 min and then analysed for D-glucose by the hexokinase/D-glucose 6-phosphate dehydrogenase method[16]. The absorbance of the NADPH formed was measured at 340 nm.

Beta-amylolysis limit (β-limit). — An aliquot (0.1 mL) of the stock solution was diluted with 0.1M sodium acetate buffer (2.4 mL, pH 4.8). A portion (1.0 mL) of this solution was treated with a diluted solution of the purified beta-amylase preparation (50 μL, 2.5 U). The mixture was incubated at 23° for 3 h, then boiled for 3 min, and cooled to room temperature. The pH was adjusted to 6.6 with 0.5M KOH (85 μL) and the solution was diluted to 2 mL with 0.1M citrate buffer (pH 6.6) containing 0.02M *N*-acetylcysteine. A portion (0.25 mL) was diluted with 1 vol. of the citric acid buffer before the addition of α-D-glucosidase (25 μL). The D-glucose formed from maltose was measured enzymically by the hexokinase/D-glucose 6-phosphate dehydrogenase method[17], using appropriate controls.

Phosphorolysis limit (ϕ-limit). — To an aliquot (0.7 mL) of the stock solution were added 1.1M sodium phosphate buffer (0.07 mL, pH 6.8) and 2.8mM EDTA (0.035 mL; di-sodium salt), followed by a freshly prepared aqueous solution (0.175 mL, 5.25 U) of phosphorylase. After incubation for 20 h at room temperature, the mixture was boiled (3 min), cooled to room temperature, and centrifuged. A portion (0.15 mL) of the supernatant solution was diluted to 1.5 mL with 0.75M triethanolamine buffer (pH 7.6) containing 10mM $MgSO_4$, and the ϕ-limit was assayed enzymically by analysing the D-glucose 1-phosphate, using phosphoglucomutase and D-glucose 6-phosphate dehydrogenase[18].

Phosphorolysis–beta-amylolysis limit (ϕ,β-limit). — The pH of the supernatant solution (0.2 mL) of a sample treated with phosphorylase *a* was adjusted to 4.8 with 0.2M acetic acid (70 μL) and diluted to 1.0 mL with 0.1M sodium acetate buffer (pH 4.8). Beta-amylolysis was then performed as described above. The maltose formed was degraded to D-glucose with α-D-glucosidase, which was assayed simultaneously with the D-glucose 1-phosphate formed during phosphorolysis, using the procedure for the determination of maltose described by Beutler[17], but modified so that 10 μL of phosphoglucomutase was added in addition to hexokinase and D-glucose 6-phosphate dehydrogenase. The ϕ,β-limit was then calculated from the total concentration of NADPH derived from the conversion of both maltose and D-glucose 1-phosphate.

Determination of non-reducing end-groups. — Rapid Smith degradation[19] was used to determine the concentration of non-reducing end groups in the amylopectin and the alpha-dextrins. The glycerol formed was determined using glycerol kinase and glycerol 3-phosphate dehydrogenase[19,20]. The total carbohydrate in these samples was determined by the amyloglucosidase method[16].

RESULTS

The alpha-dextrins studied were produced from amylopectin (waxy-maize starch) by hydrolysis with the alpha-amylase of *B. subtilis* and isolated[14] by fractional precipitation with methanol. Mixture I contained alpha-dextrins* obtained

*The individual alpha-dextrins were detected as peaks in gel filtration chromatograms and were designated dI-dV, cI-cVI, and bI-bV, in order of increasing molecular weight[14].

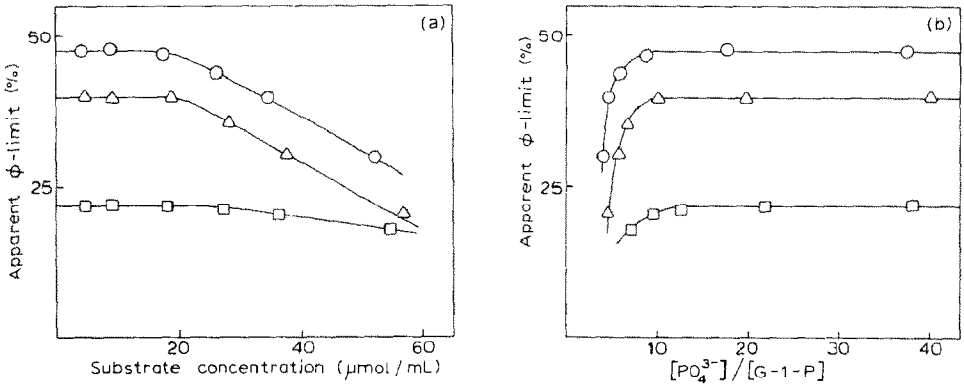

Fig. 1. Apparent phosphorolysis limit obtained with waxy-maize amylopectin (○), and mixtures I (△) and II (□) of alpha-dextrins produced after alpha-amylolysis for 60 and 210 min, respectively, as a function of (*a*) substrate concentration and (*b*) $[PO_4^{3-}]/[G\text{-}1\text{-}P]$.

after alpha-amylolysis for 60 min, and the main components are summarised in Table I together with the d.p. values previously reported[14].

After alpha-amylolysis for 210 min, the branched alpha-dextrins were precipitated with 5 vol. of methanol[14] to give mixture II, details of which are presented in Table II. The oligosaccharides (mixture III) that were not precipitated by methanol consisted almost exclusively of maltohexaose (Table II).

The reaction catalysed by rabbit muscle phosphorylase *a* is reversible and the equilibrium is strongly dependent[13] on the ratio of the concentrations of inorganic phosphate and D-glucose 1-phosphate ($[PO_4^{3-}]/[G\text{-}1\text{-}P]$). The extent of degradation with different concentrations of substrate and $[PO_4^{3-}]$ 78.6mM is shown in Fig. 1a. Below a certain concentration of substrate, each phosphorolysis limit (ϕ-limit) became constant and the value was dependent on the substrate used.

TABLE I

DATA FOR THE PRODUCTS OF ALPHA-AMYLOLYSIS OF AMYLOPECTIN FOR 60 MIN

Fraction[a]	*Composition*[b]	*D.p.*[c]	*φ-Limit (%)*	*β-Limit (%)*	*φ,β-Limit (%)*
Amylopectin			47	55	57
I	bII-cIII		40	48	54
7.1.1	bV-bIII	1550	40	48	53
7.1.2	bII-bI	810	37	47	50
7.2.1	bI-cVI	625	37	45	51
7.2	cVI-cV	515	41	48	54
7.3	cV-cIV	325	40	47	52
8.2	cIII	191	39	49	53
8.3	cII-cI	127	41	53	55
9	cII-dV	95	40	51	55

[a]See ref. 14. [b]Intermediate alpha-dextrins constituting the main part of the fraction (see ref. 14). [c]Determined[14] by gel filtration.

TABLE II

DATA FOR THE PRODUCTS OF ALPHA-AMYLOLYSIS OF AMYLOPECTIN FOR 210 MIN

Fraction[a]	*Composition*[b]	*D.p.*[c]	*ϕ-Limit (%)*	*β-Limit (%)*	*ϕ,β-Limit (%)*
II	cII-dV		22	35	38
11.2	cV-cII	193	20	30	38
11.3	cIII	165	16	30	34
12.1	cII	117	17		35
11.4	cII	115	19	31	37
11.5	cI	92	22	33	38
12.2	dV	75	23	37	40
12.3	dIV	67	26	38	42
13	dIV	67	26	40	43
III	dI	6	36	87	97

[a]See ref. 14. [b]Intermediate alpha-dextrins constituting the main part of the fraction (see ref. 14). [c]Determined[14] by gel filtration.

The $[PO_4^{3-}]$ at the end of the reaction was calculated by subtraction of the final [G-1-P] from the initial $[PO_4^{3-}]$. The apparent ϕ-limit was then plotted against the ratio $[PO_4^{3-}]$/[G-1-P] (Fig. 1b) and, regardless of the substrate used, the maximum value was obtained when the ratio was ~10. At lower final ratios, the reaction reached equilibrium before the maximum ϕ-limit was obtained. Therefore, it is important to ensure that the $[PO_4^{3-}]$/[G-1-P] ratio is high enough at the end of the reaction. If the production of D-glucose 1-phosphate is high, the lower concentration of substrate can be used.

The amylopectin had a ϕ-limit of 47% (Fig. 1a and Table I), which is higher than previously reported values (43%[12] and 41%[21]). For mixture I, the ϕ-limits were lower than that for amylopectin. Each fraction had a ϕ-limit of 39 ±2% (Table I). The ϕ-limit of alpha-dextrins in mixture II was much lower (Table II). Fractions 11.4–13 constituted a series with increasing ϕ-limits *versus* decreasing molecular weight ranging from 19% to 26%.

The beta-amylolysis limit (β-limit) of the amylopectin was 55% (Table I) when measured enzymically using the $NADP^+$-coupled reactions, which accords with the value (53%) reported by Walker and Whelan[12] but is lower than that (58%) reported by other authors[22–24]. However, when the reducing groups were analysed by the Nelson method[25] before and after hydrolysis with acid, β-limit values for amylopectin of 58–62% were obtained. The total content of carbohydrate analysed by this method after the hydrolysis with acid was 84% of the weight of the sample, whereas the enzymic method gave a value of 89%.

The β-limit values of the alpha-dextrins bV-cIII in mixture I were 47 ±2%. Fractions 8.3 and 9 (alpha-dextrins cII-dV), however, had slightly higher β-limits (Table I). The results for the alpha-dextrins in mixture II showed the same trend as the phosphorolysis (Table II). Fractions 11.2–11.3 had a β-limit of 30%, whereas

11.4–13 constituted a series with increasing β-limits. The value (10%) for 12.1 is not reported in Table II since it was probably an artefact.

Addition of beta-amylase to the ϕ-limit dextrin gave the ϕ,β-limit, which was always higher than the β-limit. This difference for the amylopectin was only 2%, but was ~5% for other fractions. Fraction 12.1 was susceptible to beta-amylase after phosphorolysis.

Mixture III, which was shown[14] to be mainly maltohexaose, had a ϕ-limit of 36% (Table II), indicating the loss of two D-glucosyl residues. The residual maltotetraose was hydrolysed completely by beta-amylase.

The average chain-length ($\overline{c.l.}$) in amylopectin and its hydrolysis products can be calculated from the proportion of non-reducing units determined by (*a*) Smith degradation[19] and enzymic assay of the resulting glycerol[19,20], and (*b*) beta-amylolysis of the ϕ-limit dextrins and assay of the maltose liberated*.

The values of $\overline{c.l.}$ for the alpha-dextrins in mixtures I and II, shown in Tables III and IV, agree. The amylopectin had $\overline{c.l.}$ values of 21.3 and 21.7 when measured by methods (*a*) and (*b*), respectively. Values of $\overline{c.l.}$ reported[12,21,24,26–29] for waxy-maize starch vary between 18.6 and 24.

The $\overline{c.l.}$ of the alpha-dextrins in mixture I averaged 14.9 when measured enzymically. There was a slight decrease in $\overline{c.l.}$ in the fractions of lower d.p. (Table III). The $\overline{c.l.}$ of the alpha-dextrins in mixture II were 11.1 on average and were fairly similar (Table IV).

TABLE III

CHAIN NUMBER AND CHAIN LENGTH OF THE PRODUCTS OF ALPHA-AMYLOLYSIS OF AMYLOPECTIN FOR 60 MIN

Fraction	*Chain number*[a]	$\overline{C.l.}$ *Smith*[b]	$\overline{C.l.}$ *Enzymic*[c]	$\overline{E.c.l.}$ ϕ[d]	$\overline{E.c.l.}$ β[e]	$\overline{I.c.l.}$ β[f]
Amylopectin		21.3	21.7	14.3	13.8	6.4[g]
I			14.0	9.6	8.7	4.3[g]
7.1.1.	98.1		15.8	10.4	9.6	5.3
7.1.2	47.5	14.5	15.7	9.9	9.4	5.4
7.2.1	41.3		15.1	9.6	8.8	5.5
7.2	32.5	17.3	15.8	10.5	9.5	5.5
7.3	20.5	18.9	15.8	10.3	9.5	5.6
8.2	13.6		14.0	9.4	8.8	4.6
8.3	9.1		14.0	9.7	9.4	4.2
9	7.1		13.4	9.4	8.8	4.4
Mean[h]			14.9	9.9	9.2	5.1

[a]Calculated as d.p./$\overline{c.l.}$. [b]Determined by Smith degradation. [c]Determined from the maltose liberated after beta-amylolysis of ϕ-limit dextrins. [d]Calculated from the ϕ-limit value, using equation *2*. [e]Calculated from the β-limit value, using equation *1*. [f]Calculated with equation *3*, using the value of $\overline{e.c.l.}$ obtained from the β-limit results. [g]Calculated with equation *4*. [h]Mean values of fractions 7.1.1-9.

*Theoretically, it should be possible to assay maltose in the presence of D-glucose 1-phosphate, using the α-D-glucosidase method. However, the enzyme preparation used showed a little activity toward D-glucose 1-phosphate, and it was necessary to hydrolyse the maltose and the D-glucose 1-phosphate.

TABLE IV

CHAIN NUMBER AND CHAIN LENGTH OF THE PRODUCTS OF ALPHA-AMYLOLYSIS OF AMYLOPECTIN FOR 210 MIN

Fraction	*Chain number*[a]	$\overline{C.l.}$ *Smith*[b]	$\overline{C.l.}$ *Enzymic*[c]	$\overline{E.c.l.}$ ϕ[d]	$\overline{E.c.l.}$ β[e]	$\overline{I.c.l.}$ β[f]
II			12.5	6.7	6.3	5.2[g]
11.2	17.7		11.0	6.2	5.3	5.0
11.3	14.8	14.4	11.2	5.8	5.4	5.2
12.1	10.3		11.3	5.9		
11.4	10.1		11.4	6.2	5.6	5.4
11.5	8.1		11.3	6.5	5.7	5.4
12.2	6.6		11.4	6.7	6.3	5.0
12.3	6.4	10.4	10.5	6.8	5.9	4.5
13	6.3	10.4	10.6	6.8	6.2	4.2
III	0.9	6.2	6.5[h]			
Mean[i]			11.1	6.4	5.8	5.0

[a]Calculated as d.p./$\overline{c.l.}$. [b]Determined by Smith degradation. [c]Determined from the maltose liberated after beta-amylolysis of ϕ-limit dextrins. [d]Calculated from the ϕ-limit value, using equation *2*. [e]Calculated from the β-limit value, using equation *1*. [f]Calculated with equation *3*, using the value of $\overline{e.c.l.}$ obtained from the β-limit results. [g]Calculated with equation *4*. [h]The maltose formed by beta-amylolysis of the ϕ-limit dextrin (maltotetraose) was divided by 2. [i]Mean values of fractions 11.2-13.

For mixture III in Table IV, the main ϕ-limit dextrin is maltotetraose[13].

It is possible to calculate[30,31] the average number of chains in each alpha-dextrin as d.p./$\overline{c.l.}$. As expected, the number of chains decreased with decreased d.p. (Tables III and IV). However, for a particular number of chains, the fractions in Table III had a higher d.p. than those in Table IV.

The average length of the external chains ($\overline{e.c.l.}$) can be calculated, using the β-limit, as

$$\overline{e.c.l.} = \overline{c.l.} \times (\%\ \beta\text{-limit}/100) + 2. \qquad 1$$

If phosphorylase *a* leaves four D-glucosyl residues next to a branch point[12], it should also be possible to calculate the $\overline{e.c.l.}$ from the ϕ-limit, using equation *2*.

$$\overline{e.c.l.} = \overline{c.l.} \times (\%\ \phi\text{-limit}/100) + 4. \qquad 2$$

$\overline{E.c.l.}$ derived for amylopectin using the β-limit was 13.8, which was 0.5 lower than the value calculated from the ϕ-limit. The products of hydrolysis obtained after alpha-amylolysis had shorter external chains. The $\overline{e.c.l.}$ was almost identical within each series of alpha-dextrin fractions. The mean value for alpha-dextrins in mixture I, using the β-limit (Table III), was 9.2 which was 0.7 lower than that calculated from the ϕ-limit. The mean value for alpha-dextrins in mixture II was 5.8, which again was 0.6 lower than that calculated from the ϕ-limit (Table IV). There was a trend of a slight increase in $\overline{e.c.l.}$ with lower d.p.

The number of internal chains is one less than the number of external chains. Therefore, the average length of the internal chains ($\overline{\text{i.c.l.}}$) can be calculated as

$$\overline{\text{i.c.l.}} = \frac{(\overline{\text{c.l.}} - \overline{\text{e.c.l.}}) \times \text{chain number}}{\text{chain number} - 1} - 1 \qquad 3$$

If the number of chains is large (or if it is unknown), the approximation *4* can be used.

$$\overline{\text{i.c.l.}} = \overline{\text{c.l.}} - \overline{\text{e.c.l.}} - 1. \qquad 4$$

In Tables III and IV, $\overline{\text{i.c.l.}}$ has been calculated using the $\overline{\text{e.c.l.}}$ value derived from the β-limits. The internal chains in the alpha-dextrins were slightly shorter than in the original amylopectin. The mean value for the alpha-dextrins in Tables III and IV were essentially the same.

DISCUSSION

The external and internal chains in a dextrin are shown in Fig. 2a. It is generally accepted that amylopectins contain an equal number of external chains with even and odd numbers of D-glucosyl residues. The structures in Fig. 3a show that β-limit dextrins, on average, have two D-glucosyl residues on the external

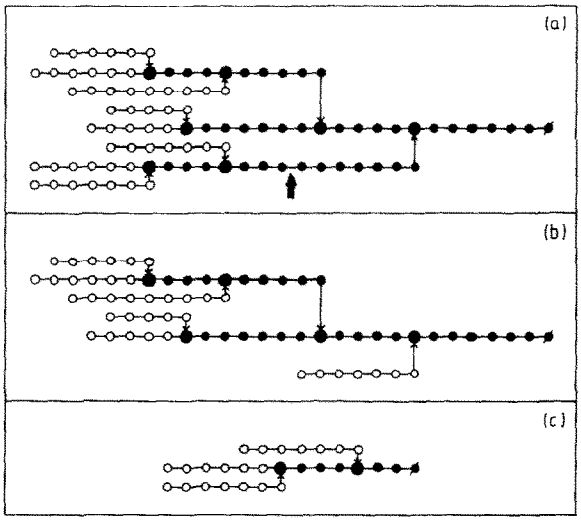

Fig. 2. Hypothetical structures of alpha-dextrins obtained by the action of *B. subtilis* alpha-amylase on amylopectin. The larger dextrin in (a) can be hydrolysed further at the point indicated by a thick arrow to give alpha-dextrins (b) and (c): —, (1→4) linkage; ↓, (1→6) linkage; D-glucosyl residues in external chains (○), and internal chains (●), branch points (●), and at the reducing ends (●). A-Chains and B-chains are attached by (1→6) linkages with A-chains unsubstituted and B-chains substituted by other A- or B-chains, and the C-chain carries the sole reducing-end group[37].

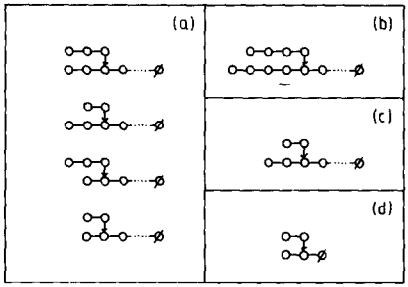

Fig. 3. Products obtained from amylopectin: (a) β-limit dextrins from the action of sweet-potato beta-amylase[11]; (b) ϕ-limit dextrin from the action of rabbit muscle phosphorylase a[12]; (c) ϕ,β-limit dextrin from the action of beta-amylase on the ϕ-limit dextrin; (d) the smallest, singly branched, limit dextrin produced by *B. subtilis* alpha-amylase[8,9].

chains. Therefore, the $\overline{\text{e.c.l.}}$ can be calculated from equation *1*. The average $\overline{\text{e.c.l.}}$ in the β-limit dextrin is 2 only if the ratio of A- and B-chains is 1. This ratio was not measured but was assumed to be 1.

The structure of the ϕ-limit dextrin in Fig. 3b was proposed by Walker and Whelan[12]. Their experimental data, however, did not indicate the length of the outer portion of the B-chain. The use of equation *2* to give the $\overline{\text{e.c.l.}}$ of a dextrin is based on the structure in Fig. 3b. Tables III and IV show that the $\overline{\text{e.c.l.}}$ calculated from the ϕ-limit with equation *2* is higher than that obtained using the β-limit. The difference for amylopectin was 0.5 D-glucosyl residue, and the differences of the mean values of the alpha-dextrins were 0.7 and 0.6 D-glucosyl residues. Thus, the ϕ-limit dextrin is 0.5 D-glucosyl residue shorter than proposed[12]. In the structure in Fig. 4a, the average external chain-length (assuming an equal number of A- and B-chains) is 3.5 D-glucosyl residues. The A-chain still has 4 residues as proposed[12], but the B-chain has only 3 residues. This structure is analogous to the largest and smallest possible β-limit dextrins in Fig. 3a, in which the B-chain also is one D-glucosyl residue shorter than the A-chain. Takeda and Hizukuri[32], who studied the action of rabbit muscle phosphorylase a on phosphorylated (1→4)-α-D-glucans, showed that the enzyme also leaves three D-glucosyl residues attached to a 6-phosphorylated residue on the non-reducing side. For many enzymes, 6-phosphorylated residues act as barriers to hydrolysis in a way similar to the branch points in amylopectin[32].

Another indication of the structure of the ϕ-limit dextrin is the fact that the ϕ,β-limit was always larger than the β-limit (Tables I and II). From the structure of the ϕ,β-limit dextrin in Fig. 3c, which is based on the ϕ-limit dextrin in Fig. 3b and resembles the average β-limit dextrin, a ϕ,β-limit equal to the β-limit would have been expected. The new ϕ-limit dextrin structure, which still allows beta-amylase to hydrolyse one maltosyl residue from each external chain, gives the ϕ,β-limit structure depicted in Fig. 4b. This structure is identical to the shortest possible β-limit dextrin with an $\overline{\text{e.c.l.}}$ of only 1.5 residues (Fig. 3a). This situation could explain why the ϕ,β-limit was always higher than the β-limit.

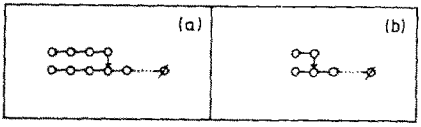

Fig. 4. Proposed structures of (a) ϕ-limit dextrin obtained by the action of phosphorylase *a* on amylopectin and the intermediate alpha-dextrins; (b) ϕ,β-limit dextrin obtained by the action of beta-amylase on the ϕ-limit dextrin.

The use of rabbit muscle phosphorylase *a* and sweet-potato beta-amylase to determine the number of non-reducing ends has to be restricted to alpha-dextrins possessing external chains with a length of at least 4 D-glucosyl residues on the A-chains and 3 residues on the B-chains. As a rule, a higher ϕ,β-limit than β-limit should indicate that this is so, although the existence of short external chains together with longer chains cannot be ruled out.

Kainuma and French[33] showed that a single D-glucosyl residue in the external A-chain will make the (1→4) linkage next to the branch point in the external B-chain susceptible to beta-amylase if the B-chain contains an even number of D-glucosyl residues. However, according to the structure proposed in Fig. 4a, the ϕ-limit dextrin contains an odd number of residues in the external B-chain. In addition, the probability of obtaining alpha-dextrins with such short external chains after some hours of hydrolysis is small. The smallest, singly branched, limit dextrin produced by the alpha-amylase of *B. subtilis* has two D-glucosyl residues on the external A-chain[8,9] (Fig. 3d) and is produced only after prolonged hydrolysis.

Average chain-lengths determined by Smith degradation correlated fairly well with the $\overline{\text{c.l.}}$ determined after enzymic degradation (Tables III and IV). The variation in the results with the former method was much higher, being of the order of ±3 D-glucosyl residues. In the Smith-degradation procedure, it is difficult to control exactly the extent of periodate oxidation[19]. With the enzymic method, a variation in the $\overline{\text{c.l.}}$ of ±1 D-glucosyl residue was obtained. Despite the limitations of the method, it gives precise results and is easy to perform.

The molecular weight of an alpha-dextrin depends on the time of alpha-amylolysis, but the chain number is a characteristic constant which can be used to identify the individual dextrins. Thus, the chain numbers in Tables III and IV can be compared with the composition of the fractions as indicated by gel filtration (Tables I and II). For instance, fraction 8.2, in which the alpha-dextrin cIII preponderates, had a chain number of 13.6, which correlates with the lower-molecular-weight dextrins in fraction 11.3 that also contains cIII.

When the chain number of an alpha-dextrin is constant, the only reasonable way to reduce its molecular weight is by shortening the external chains. Table IV shows that the $\overline{\text{e.c.l.}}$ of the dextrins in mixture II were shorter than those in mixture I (Table III). The maltohexaose (dI), present in mixture III, has been suggested to be produced from the external chains[1,3,10].

Alpha-dextrins produced after alpha-amylolysis for a particular time had almost identical $\overline{\text{e.c.l.}}$ values, regardless of their molecular weight or chain number.

This finding suggests that hydrolysis of amylopectin by the alpha-amylase of *B. subtilis* involves two independent processes. In the first process, attack on the internal B-chains occurs, giving alpha-dextrins larger than maltohexaose. These dextrins were suggested[10] to contain branch points grouped into clusters in accordance with the cluster model[34]. The various alpha-dextrins will contain intact clusters, parts of clusters, or two or several clusters interconnected by B-chains. These B-chains have been suggested to contain at least 40 D-glucosyl residues[35], which may be attacked easily by the enzyme in early stages of the hydrolysis[10]. The second process releases maltohexaose from the external chains and has the same rate for the amylopectin and alpha-dextrins, which is dependent therefore on the total concentration of external chains.

Because of these two simultaneous processes, the $\overline{\text{c.l.}}$, $\overline{\text{e.c.l.}}$, and $\overline{\text{i.c.l.}}$ of the alpha-dextrins are expected to decrease continuously as alpha-amylolysis proceeds. However, this situation did not seem to be true for the lower-molecular-weight dextrins in mixture II. Table II shows an increase in the β-, ϕ-, and ϕ,β-limits, with lower molecular weight. A small increase in $\overline{\text{e.c.l.}}$ for these samples is shown in Table IV.

Fig. 2 explains this result. The hypothetical alpha-dextrin (Fig. 2a) has characteristics (Table V) similar to those of fraction 11.5, which contains the alpha-dextrin cI (Tables II and IV). The site of attack by the alpha-amylase occurs at an internal chain as indicated, which produces two new alpha-dextrins. The dextrin in Fig. 2b is comparatively large and the residual right-hand part of the cleaved B-chain is now a new external chain. The characteristics of this dextrin (Table V) include a significant increase in the beta-amylolysis and phosphorolysis limits, a slight increase in $\overline{\text{e.c.l.}}$, and a resemblance to fractions 12.2 and 12.3.

The smaller dextrin produced (Fig. 2c) has no new external chains and is characterised by marked increases in the β- and ϕ-limits, since the resistant part of the molecule is much smaller.

It has been suggested that amylopectin is composed of units of clusters of defined sizes and structures[10,36]. An isolated unit should have characteristics, such

TABLE V

CHARACTERISTICS OF THE HYPOTHETICAL ALPHA-DEXTRIN STRUCTURES IN FIG. 2

Parameter	*Fig. 2a*	*Fig. 2b*	*Fig. 2c*
A:B chain ratio	1.67:1	2.0:1	2.0:1
Chain number	8	6	3
D.p.	96	68	28
$\overline{\text{C.l.}}$	12.0	11.3	9.3
$\overline{\text{E.c.l.}}$	6.4	6.3	6.7
$\overline{\text{I.c.l.}}$	5.4	5.0	3.0
β-Limit (%)	33	35	43
ϕ-Limit	23	24	32
ϕ,β-Limit (%)	39	41	54

as β-, ϕ-, and ϕ,β-limits and the length of the external chains, similar to those of the intact macromolecule. The first process in the alpha-amylolysis will liberate unit clusters. Because of the simultaneous second process, the e.c.l. of the intermediate alpha-dextrins will decrease continuously and therefore not be comparable to the amylopectin. However, since the second process appears to continue at a constant rate regardless of the size of the alpha-dextrins, the characteristics of the products can be compared at fixed hydrolysis times. The characteristics of the alpha-dextrins in Table I were almost identical for all the fractions down to cIII. Samples 8.3 and 9, containing dextrins cII-dV, had slightly higher hydrolysis limits. In Table II, fractions 11.2–12.1 were similar, whereas there was a continuous increase in hydrolysis limits for fractions 11.4–13 which contained dextrin cII and smaller dextrins. On the basis of these results, it is suggested that the unit cluster of the waxy-maize amylopectin occurs among the alpha-dextrins of a size near that of cI.

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