Carbohydrate Research, 243 (1993) 307–321
Elsevier Science Publishers B.V., Amsterdam

Structures of the amylopectins of waxy, normal, amylose-extender, and wx : ae genotypes and of the phytoglycogen of maize

Suck-Hun Yun and Norman K. Matheson *

Department of Agricultural Chemistry, The University of Sydney, NSW 2006 (Australia)

(Received July 28th, 1992; accepted November 6th, 1992)

ABSTRACT

Average chain lengths and beta-amylolysis limits have been determined for the waxy and ae/wx genotypes of mature maize starch, and for the amylopectin fractions of normal and amylose-extender starches (prepared by precipitation with concanavalin A), rabbit-liver glycogen, phytoglycogen, and waxy rice starch. All amylopectin samples had similar A:B chain ratios of >1 and the two glycogens had ratios of <1. This finding led to average frequencies of substitution of B chains over the whole molecule of >2 for the amylopectins and <2 for the glycogens. An equation for the number of tiers for a molecule with various frequencies of substitution of B chains and chain lengths has been used to determine the effect of variation in average frequency of branching and average chain length on structure.

INTRODUCTION

The structure of $(1 \rightarrow 4)(1 \rightarrow 6)$-linked α-D-glucans like amylopectin, phytoglycogen, and glycogen can be described by several characteristics which include the average chain length (CL), the average external chain length (ECL) as estimated from the beta-amylolysis limit and the CL, the internal chain length (ICL), and the A:B chain ratio [where A chains contain only $(1 \rightarrow 4)$ linkages and B chains also contain $(1 \rightarrow 6)$ linkages][1,2]. Determination of these parameters for the amylopectins in starches that have an amylose component (e.g., normal and amylose extender) requires prior removal of the amylose. These starches can be fractionated by complexing with 1-butanol[3] and/or by gel permeation chromatography[4] (GPC), but there are problems associated with incomplete fractionation, particularly of amylose-extender starches. The use of concanavalin A to precipitate branched structures has been described[5–7]. Most A:B chain ratios have been determined for $(1 \rightarrow 4)(1 \rightarrow 6)$-linked α-D-glucans that have no unbranched com-

* Corresponding author.

0008-6215/93/$06.00 © 1993 – Elsevier Science Publishers B.V. All rights reserved

ponent (glycogen, phytoglycogen, and waxy starches), but values have also been found for two normal rice-amylopectin fractions prepared by complexing with 1-butanol[8].

We now describe the measurement of the A:B chain ratios, CL values, and other characteristics of the amylopectins separated by precipitation with concanavalin A from the normal and amylose-extender starches of mature maize seeds, as well as some other $(1 \rightarrow 4)(1 \rightarrow 6)$-linked α-D-glucans, namely, ae/wx, waxy maize, and waxy rice starches, phytoglycogen, and rabbit-liver glycogen. General expressions have been derived for the calculation of the CL values when the A:B chain ratio differs from 1.

DISCUSSION

In the determination of the A:B chain ratios, beta-amylolysis limits were obtained by analysis of the maltose released by the Somogyi–Nelson reagent and by GPC on Fractogel TSK HW 40(S). A typical GPC elution pattern is shown in Fig. 1A. GPC on Bio-Gel P-2 and determination of the eluted carbohydrates with the orcinol–sulphuric acid reagent has been described[8]. The beta-amylolysis limits obtained by these methods for the seven α-D-glucans are shown in Table I. The normal and amylose-extender maize amylopectins were prepared by fractionation with concanavalin A of starches obtained from mature whole grains. There was reasonable agreement between the two methods (Table I) and no evidence from chromatography for the production of glucose. The CL values were determined by reduction of cupric ion after debranching with isoamylase. The A:B chain ratio is usually calculated from the amounts of maltose and maltotriose released on debranching of the beta-limit dextrin and there have been several approaches. The first approach[9] involved chromatography on a column of charcoal–Celite. Subsequently, quantitative PC[10], reversed-phase chromatography[11] on μ Bondapak-NH_2, and GPC on Fractogel TSK HW-40(S)[12] or Bio-Gel P-2[8] were applied. In this work, GPC on TSK HW-40(S) with detection by refractive index was used. A typical elution pattern is shown in Fig. 1B. The fraction of A chains (a) was calculated from eq. *1* and the A:B chain ratio from eq. *2*.

$$a = \frac{\%(\text{maltose} + \text{maltotriose})}{100 \times 2.5} \times \frac{\text{CL}(100 - \text{beta amylolysis limit}\%)}{100} \qquad (1)$$

$$\text{A:B chain ratio} = \frac{a}{1 - a} \qquad (2)$$

The value 2.5 in eq. *1* derives from the CL of the maltosyl and maltotriosyl stubs left of the original A chains after beta-amylolysis.

The second multiplier in the right-hand side of eq. *1* is the CL of the beta-limit dextrin (CLβ) and means that the A:B chain ratio of the original glucan could be obtained by measuring CLβ directly: the A:B chain ratios of the original glucan

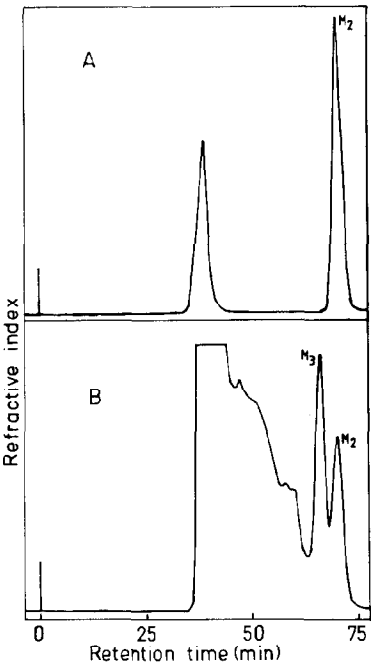

Fig. 1. GPC on TSK HW40(S) of the beta-amylolysis product of ae/wx maize starch (A) and of debranched beta-limit dextrin of n-maize amylopectin (M_2, maltose; M_3, maltotriose).

and of the beta-limit dextrin are identical. Determination of the A:B chain ratio from the maltose and maltotriose released by the debranching enzyme from the beta-limit dextrin requires that there are no B chains of < 4 glucose residues. These chains could arise if the parent glucan had B chains of < 4 glucose residues or if these could be produced on beta-amylolysis, as shown in Fig. 2. The isolation of equimolar amounts of maltose and maltotriose indicates that significant amounts

TABLE I

A:B chain ratios of $(1 \rightarrow 4)(1 \rightarrow 6)$-linked α-D-glucans

Source	Waxy maize	Maize	Maize (ae/wx)	Maize (ae)	Waxy rice	Phyto-glycogen	Glycogen
CL	18	22	28	33	20	12	14
Beta-limit [a]	52	56	54	60	53	32	43
Beta-limit [b]	53	55	54	57	53	30	43
G_2+G_3	15.9	13.9	10.7	10.8	15.2	14.4	12.7
$G_3:G_2$	1.01	0.95	1.00	0.95	1.01	1.10	1.02
CL_β [c]	9	10	13	13	9	8	8
a [d]	0.56	0.54	0.55	0.57	0.56	0.45	0.42
A:B	1.3	1.2	1.2	1.2	1.2	0.9	0.7

[a] By copper reduction. [b] By GPC on Fractogel-HW40(S). [c] Calculated for $CL \times (100 - \beta\text{-limit}) \div 100$. [d] Fraction of A chains.

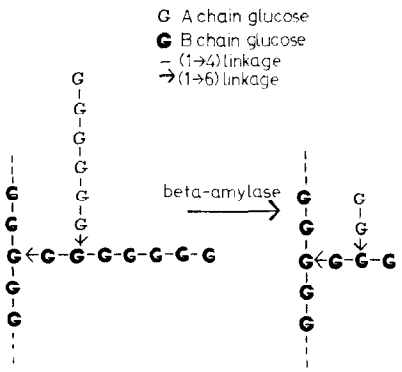

Fig. 2. Production of B chains of three glucosyl units after beta-amylolysis.

of maltotriose have not been derived from B chains. Debranching of the beta-limit dextrin of shellfish glycogen by isoamylase plus pullulanase produced more maltotriose than by pullulanase alone[13]. This result was interpreted as revealing B chains with a length of three glucose residues. However, on a molar basis, equal amounts of maltose and maltotriose were produced only by the combination of enzymes; pullulanase alone produced less than the molar equivalent of maltotriose. In a study of three glycogens and phytoglycogen[14] that had been debranched with isoamylase and the products analysed by reversed-phase chromatography, significant amounts of maltotriose were reported (3.6–9.3%). Thus, chains of three glucose residues were present; these could be A or B chains. The percentage of maltotriose detected in one sample was almost as high as that usually detected after debranching of beta-limit dextrins. When rabbit-liver glycogen was debranched with either isoamylase or isoamylase plus pullulanase and the products were fractionated by GPC on TSK HW 40(S), no maltotriose could be detected. On the other hand, small peaks corresponding to maltotriose (0.7%) and maltose (0.3%) were detected after phytoglycogen had been debranched. Also, the ratio of maltotriose to maltose released on debranching of the beta-limit dextrin was significantly different from 1. PC of the products in a debranched sample of phytoglycogen revealed maltotriose and maltose. Under similar conditions, the debranching of rabbit-liver glycogen gave no maltotriose or maltose.

The A : B chain ratios determined for glycogen, phytoglycogen, and waxy maize and rice starches (Table I) were generally similar to those reported[1,2,8–12,15–17]. The values for glycogen and phytoglycogen were < 1 and those for amylopectin were > 1. The ratios for normal and waxy maize amylopectins were similar. The ratios for waxy rice starch[8] and two normal amylopectins, prepared by complexing with 1-butanol–pentanol, were > 1. The A : B chain ratios for the ae : wx and amylose-extender genotypes of maize were similar to those for the waxy and normal amylopectins (Table I), which showed the similarity in the ratio for all four genotypes.

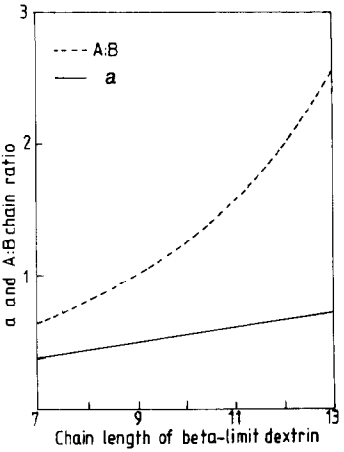

Fig. 3. Effect of differing chain length of a beta-limit dextrin on the fraction of A chains (*a*) and the A:B chain ratio (for n-amylopectin).

The introduction of column chromatographic techniques has allowed accurate determination of maltose and maltotriose released from the beta-limit dextrin by debranching. However, accurate measurement of CL is more difficult. Various procedures for chain-length estimation have given different values[18] and the experimental error for a particular method is ± 1 glucose residue[1]. Variation in the value of CL has a greater effect on the calculation of the A:B chain ratio for amylopectins than for glycogen. Although the CL (as well as the amount of maltose plus maltotriose released) is related linearly to the fraction of A chains (*a* in eq. *1*), the relationship between the A:B chain ratio and CL is non-linear (Fig. 3). This situation is a consequence of the nature of eq. *2*. Similar increments in the value of *a* have an increasing effect on the A:B chain ratio as CL increases. This effect is illustrated for n-maize–amylopectin in Fig. 3. In the plot of eq. *2* (between *a* values of 0 and 1), the A:B chain ratio rises from 0 to 1 as *a* increases from 0 to 0.5, but from 1 to infinity as *a* increases from 0.5 to 1.

In this study, CL was determined by the copper-reducing power of debranched glucan. The reducing power of a series of maltosaccharides with dp 1–7 showed no differences within experimental error for mol equiv, in agreement with earlier findings[19]. In the determination of CL for the debranched glucans, maltohexaose was used as the standard in order to minimise any effect of dp on reducing power. The glucan content was determined with the phenol–sulphuric acid reagent.

From the CL and the beta-amylolysis limit, and assuming that the A:B chain ratio is 1, other parameters can be calculated[1,2]. The average external chain length (ECL) can be calculated from eq. *3*

$$\mathrm{ECL} = \mathrm{CL} \times \frac{\text{beta-limit}\%}{100} + 2 \qquad (3)$$

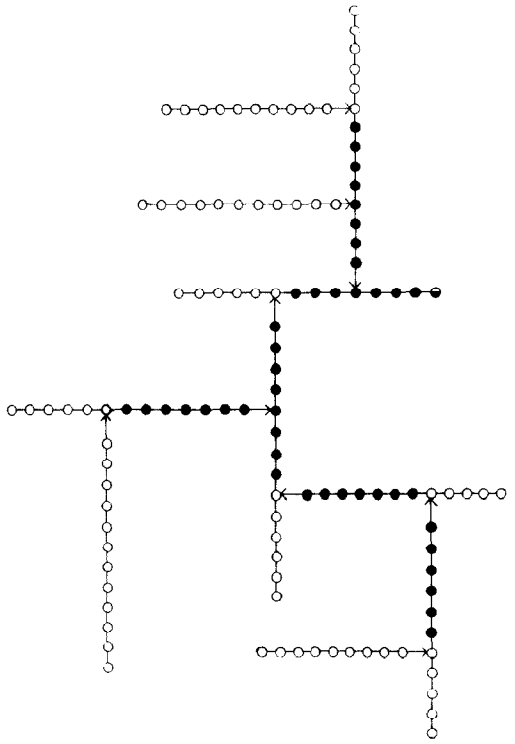

Fig. 4. Core chains in a (1 → 4)(1 → 6)-linked glucan: glucosyl units, ○ ● ◒; reducing end, ◒; core chain unit, ●; (1 → 4) linkages, ●——● ○——○; (1 → 6) linkages, ○——→○ ○——→●.

and the average internal CL (ICL) from eq. *4*

$$ICL = CL - ECL - 1 \quad (4)$$

The value 2 in eq. *3* is the mean of the CLs of the outer stubs left after beta-amylolysis i.e., 2.5 for A chains (with equal numbers of 2- and 3-unit glucosyl stubs) and 1.5 for B chains (with equal numbers of 1- and 2-unit glucosyl stubs).

General expressions for ECL and ICL for any A : B chain ratio can be derived. Most determinations of the A : B chain ratios of amylopectins have indicated values somewhat higher than 1 and < 1 for glycogens.

CL from the outermost branch point to the reducing end of the (1 → 4) linkages [i.e., ignoring any other chains that are linked (1 → 6) thereto and symbolised as CCL (core chain length)] can be calculated from eq. *5*

$$CCL = \frac{CL_{\beta} - 2.5}{b} \quad (5)$$

where b is the fraction of B chains. The core chains do not include the glucosyl unit that carries the outermost branch and they are illustrated in Fig. 4. The CCL is the number of glucosyl units from the outermost black circle of a (1 → 4)-linked

chain to the point at which a glucosyl unit in this chain is linked to HO-6 of another chain.

Equation 5 is derived from

$$CL_{\beta} = aCL_{\beta A} + bCL_{\beta B}$$

where $CL_{\beta A}$ and $CL_{\beta B}$ are the values of CL of A and B chains in the beta-limit dextrin and a the fraction of A chains. Then, since

$$CL_{\beta A} = 2.5$$

$$CL_{\beta} = 2.5a + bCL_{\beta B}$$

but

$$CL_{\beta B} \text{ is } CCL + 2.5$$

therefore,

$$CL_{\beta} = 2.5a + bCCL + 2.5b$$

and

$$CCL = \frac{CL_{\beta} - 2.5(a + b)}{b}$$

but $a + b = 1$ so,
therefore,

$$CCL = \frac{CL_{\beta} - 2.5}{b}$$

The CL for the B chains of the beta-limit dextrin ($CL_{\beta B}$) is then CCL + 2.5.

The CL for the exterior chains can be calculated from eq. 6

$$ECL = CL - CL_{\beta} + 2.5 - b \qquad (6)$$

which can be derived from

$$CL = aCL_{A} + bCL_{B}$$

where CL_{A} and CL_{B} are the values of CL for the A and B chains.

Then, $CL = aCL_{A} + b(CCL + 1 + CL_{BE})$ where CL_{BE} is the value of CL for the B chains exterior to the outermost branch point.

Since $aCL_{A} + bCL_{BE} = ECL$ then $CL = ECL + bCCL + b$
therefore,

$$ECL = CL - bCCL - b$$

then, from the relationship between CL_{β} and CCL (eq. 5),

$$ECL = CL - CL_{\beta} + 2.5 - b$$

ICL can be calculated from eq. 7

$$ICL = bCCL - a \qquad (7)$$

TABLE II

Chain lengths of (1 → 4)(1 → 6)-linked α-D-glucans

Source	Waxy maize	Maize	Maize (ae/wx)	Maize (ae)	Waxy rice	Phyto-glycogen	Glycogen
CL	18	22	28	33	20	12	14
CLβ	9	10	13	13	9	8	8
CLβA	2.5	2.5	2.5	2.5	2.5	2.52	2.5
CCL [a]	14	17	23	25	15	10	10
ECL [a]	12	14	17	22	12	6	8
ICL [a]	6	7	10	10	6	5	5
CLβB [a]	17	19	25	28	18	12	12
F [b]	2.3	2.2	2.2	2.2	2.2	1.9	1.7

[a] Calculated from CL values to the first decimal place but expressed as whole numbers. [b] Average number of times a B chain bears a (1 → 6)-linked branch (see text).

Equation 7 can be derived from the relationship

$$\mathrm{ICL} = \frac{T_B \times \mathrm{CCL} - (T_A - 1)}{T_A + T_B - 1}$$

where T_A and T_B are the total numbers of A and B chains.

After division of numerator and denominator by the total number of chains (T), the right-hand side becomes

$$\frac{\dfrac{T_B \times \mathrm{CCL}}{T} - \left(\dfrac{T_A}{T} - \dfrac{1}{T}\right)}{\left[\dfrac{T_A}{T} + \dfrac{T_B}{T} - \dfrac{1}{T}\right]} = \frac{b\ \mathrm{CCL} - \left[a - \dfrac{1}{T}\right]}{1 - \dfrac{1}{T}}$$

as T becomes large $1/T \to 0$ therefore, eq. 7 holds.

The various chain-length values for the (1 → 4)(1 → 6)-linked α-D-glucans are shown in Table II. The total chain length increases from waxy maize amylopectin to normal to ae : wx, and confirms the qualitative results obtained[7] by debranching and GPC. The partial chain lengths for the maize amylopectins increased similarly, so that lengthening of the total CL does not lead to a lengthening of a particular part of the total chain at the expense of another. The ratio CCL : ECL is similar (1.1–1.3) for all the amylopectins and rabbit-liver glycogen. Phytoglycogen is characterised by a short ECL and thus CCL : ECL is larger (1.7) than for all the other samples, leading to a much lower beta-amylolysis limit. Small proportions of maltotriose and maltose were released on debranching of the whole polysaccharide. On the other hand, the CCL : ECL ratio for rabbit-liver glycogen was similar to that of the amylopectins and no maltosaccharide with a dp < 4 was released on debranching. Except for amylose-extender amylopectin sampled at the earliest stage of development[7], the elution profiles of debranched amylopectins from different times within genotypes were similar, which indicated that the polysaccharides from immature and mature grains had similar structures. The different values

of ICL may reflect different patterns of action for the branching enzymes involved in the conversion of (1 → 4)-linked glucan into the (1 → 4)(1 → 6)-linked glucan. Those enzymes which synthesise the amylopectins of the normal and amylose-extender genotypes require a longer (1 → 4)-linked chain than the waxy amylopectin for branching to occur. Different branching enzymes have been described[20–22]. This requirement for a longer (1 → 4)-linked glucan substrate may be a factor in the deposition of amylose in the normal and amylose-extender genotypes. The content of amylose increases with the physiological age of an organ that accumulates starch[23], and amylose is absent (or present in low proportions) at the early stages of synthesis of a starch which later contains amylose[24]. If a longer chain is required for branching to occur, then there is more opportunity for developing (1 → 4)-linked chains to escape branching and precipitate. However, the behaviour of the ae:wx mutant means that this cannot be the only factor.

The average frequency with which B chains are substituted by (1 → 6)-linked chains (F) can be calculated from eq. *8*,

$$F = \frac{1}{1-a} \tag{8}$$

which can be derived as follows. A core chain is made up of its internal chains plus the number of 6-linked glucosyl units, less the single outermost 6-linked glucosyl unit

i.e., $\mathrm{CCL} = F \times \mathrm{ICL} + (F - 1)$

therefore,

$\mathrm{CCL} + 1 = F \times \mathrm{ICL} + F$

$\mathrm{CCL} + 1 = F(\mathrm{ICL} + 1)$

$$F = \frac{\mathrm{CCL} + 1}{\mathrm{ICL} + 1}$$

therefore,

$$F = \left[\frac{(\mathrm{CL}_\beta - 2.5)}{b} + 1\right] \div \left[b\frac{(\mathrm{CL}_\beta - 2.5)}{b} - a + 1\right]$$

$$= \left[\frac{(\mathrm{CL}_\beta - 2.5)}{b} + 1\right] \div \left[b\frac{(\mathrm{CL}_\beta - 2.5)}{b} + b\right]$$

$$= \left[\frac{(\mathrm{CL}_\beta - 2.5)}{b} + 1\right] \div b\left[\frac{(\mathrm{CL}_\beta - 2.5)}{b} + 1\right]$$

$$= \frac{1}{b}$$

$$= \frac{1}{1-a} \text{(i.e. eq. 8)}$$

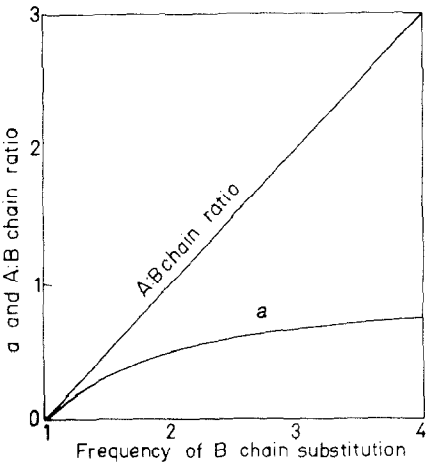

Fig. 5. Relationship between the frequency of substitution of B chains of a (1 → 4)(1 → 6)-linked α-D-glucan and the values of a and the A:B chain ratio.

The A:B chain ratio can be related to F (eq. *9*)

$$\text{A:B chain ratio} = F - 1, \tag{9}$$

which follows from

$$\text{A:B chain ratio} = \frac{a}{1-a} = \frac{1}{1-a} - 1 = F - 1$$

and the proportion of A chains in relation to F is given by eq. *10*

$$a = \frac{F-1}{F} \tag{10}$$

Hence, the A:B chain ratio is related linearly to F and non-linearly to a (Fig. 5). The average frequencies of substitution, shown in Table II, can be divided into two groups, namely, the amylopectins ≥ 2.2 and phytoglycogen and rabbit-liver glycogen ≤ 1.8. These F values are an average for the whole molecule. Debranching of amylopectin followed by GPC shows a wide range of chain lengths for the component (1 → 4)-linked chains. Sequential hydrolysis with beta-amylase, isoamylase, and beta-amylase established that the longer chains in amylopectin carry a higher number of branches[17]. This finding means that, in the cluster model that is generally accepted for amylopectin, a number of longer B chains have considerably more than two chains attached. Thus, in order to produce an average frequency of substitution of 2.3, there must be a compensating number of B chains with 2 branches or even only 1. The chain length distribution of the (1 → 4)-linked chains of glycogen is much less diverse than that of amylopectin. With a frequency of branching of 1.7, some chains must carry only one branch. The shorter chains possibly carry the lower number of branches. This arrangement would accord with

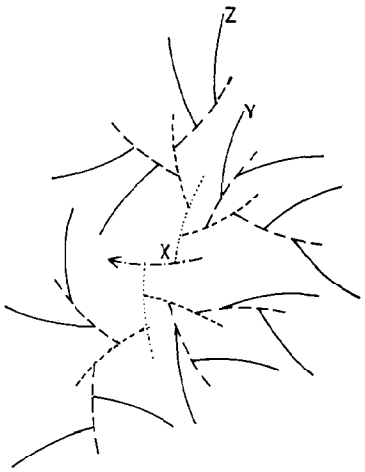

Fig. 6. Tier structure of (1 → 4)(1 → 6)-linked α-D-glucans: C chain, ·–·–·; 1st tier, ·····; 2nd tier, - - - - -; 3rd tier, — — —; 4th tier, ———; reducing end, →. For X, Y, and Z, see text.

models of the pattern of action of branching enzyme that requires a minimum length of α-(1 → 4)-linked chain.

In the idealised model shown in Fig. 6, in which both chain length and frequency of branching are uniform throughout, the number of tiers of (1 → 4)-linked chains required to reach a particular dp (assuming uniform dendritic formation as shown for this two-dimensional representation) can be calculated from the equation

$$\frac{\mathrm{MW}}{162} \times \frac{1}{\mathrm{CL}} = F^0 + F^1 + F^2 + F^3 \cdots F^T$$

where T is the number of tiers and MW the molecular weight. MW/162 is the dp. This equation can be transformed into eq. *11*

$$T = \frac{\log[1 - \mathrm{dp}(1 - F) \div \mathrm{CL}]}{\log F} \qquad (11)$$

Calculations for chain lengths of 20–25 and a frequency of 2.3 (similar to values for amylopectin) show that, for a molecular weight of 10^7, the number of tiers required is 10, whereas, for chain lengths of 12 and 14 with frequencies of 1.9 and 1.7, respectively (similar to values for phytoglycogen and glycogen), the number of tiers required are 13 and 15. Calculations of dendritic growth structure for glycogen have indicated that crowding of glucosyl units as the number of tiers increases may limit expansion[25]. For a frequency of branching of 2, it was calculated from the size of a maltosaccharide chain that the 12th tier would be the limit of attainable size. GPC indicates that the molecular size of dissolved amylopectin is higher than that of glycogen and phytoglycogen[4,26,27].

TABLE III

Effect of frequency of substitution (F) on the number of tiers (T) required to reach a MW of 10^7 with CL constant (20)

Frequency of substitution (F)	No. of tiers required (T)
1.5	18
2.0	12
2.5	9
3.0	8
4.0	7

An aspect of the model shown in Fig. 6, in which both the total and internal chains have each been drawn with uniform lengths and F is 2, is that the number of glucosyl units, counting from the linkage to the C chain to the non-reducing end of a sequence of (1 → 4)-linked chains and ending in the same number of tiers, is considerably smaller for the sequence of chains always joined closer to the (1 → 6) linkage (X to Y) than for the sequence always joined further from the (1 → 6) linkage (X to Z). Other sequences have intermediate values. Thus, different parts of the same tier are at different distances from the C chain (in terms of number of glucosyl units). For a structure with four completed tiers, as in Fig. 6, to extend all the series of α-(1 → 4)-linked chains to the same number of glucosyl units [or this value less one-third of the CL of an α-(1 → 4)-linked chain] would require, in addition to the chains shown in Fig. 6, 22 chains in the fifth tier, 12 in the sixth, and 2 in the seventh.

The average frequency of substitution is the major factor which affects the number of tiers required to reach a particular dp. For an ideal polysaccharide with a uniform CL of 20, the effect of different frequencies of substitution is shown in Table III. As F changes from 1.5 to 2.5, the number of tiers required to form a molecule with MW 10^7 decreases from 18 to 9. In contrast, changes in CL have little effect on the number of tiers required to form a structure with this MW and an F value of 2.3. Changing the CL from 10 to 35 only changes the number of tiers required from 10.8 to 9.3 (Table IV). The main effect of the longer chain lengths of amylopectin may be to produce the ellipsoidal shape. The idealised model shown

TABLE IV

Effect of CL on the number of tiers (T) required to reach a MW of 10^7 with F constant at 2.3

Chain length (CL)	No. of tiers required (T)
10	11
15	10
20	10
25	10
30	9
35	9

in Fig. 6 serves to illustrate the scale of differences that can exist in the numbers of glucosyl units between the non-reducing end of chains in the same tier and the reducing end of the molecule, and also the relative effect of chain length and frequency on the number of tiers required to produce a particular molecular weight. The actual structure of amylopectin must accommodate the variation in chain lengths which leads to a cluster structure, different degrees of substitution in chains, the possibility that some interior chains do not continue to grow (buried A chains), and also that the hydrodynamic properties (unlike glycogen) are consistent with an ellipsoidal rather than a spheroidal three-dimensional structure[1,2,15]. From its solution properties and the more limited range of (1 → 4)-linked chain lengths, the structure of glycogen is probably closer to the idealised model.

EXPERIMENTAL

Glucan samples.—The four genotypes of maize (waxy, normal, amylose-extender, and an ae/wx double mutant) were grown and starches from the mature grains prepared as described[7]. Starch from mature waxy rice (the hybrid YR71003 from the cross Calrose*4/I-geo-tse//YR140 cropped at Yanco Agricultural Institute) was also isolated by the same procedure. Phytoglycogen from sweet corn, su_1, a back-crossed sub-line of W22, was prepared from the supernatant solution which remained after centrifugation at 28000*g* of a 0.01 M Hg^{2+} extract and dissolution in Me_2SO of the product precipitated with EtOH (ref. 27). Rabbit-liver glycogen was obtained from Sigma (Type III-G8876).

Enzymes.—Beta-amylase [(1 → 4)-α-D-glucan : maltohydrolase, EC 3.2.1.2, sweet potato] and pullulanase (EC 3.2.1.41, *Aerobacter aerogenes*) were purchased from Boehringer, and isoamylase (EC 3.2.1.68, *Pseudomonas*) from Hayashibara Biochemical Laboratories (Japan).

Preparation of amylopectins.—Amylopectin from n and amylose-extender maize was fractionated as described[5,6], with the following modification to remove traces of protein. After precipitation with EtOH, the amylopectin fraction was isolated by centrifugation (1500*g*, 15 min) and a solution in Tris-buffer (20 mL, pH 8) was boiled and immediately cooled. Proteinase-K (10 U) was added, the solution was incubated for 12 h and then centrifuged (1500*g*, 10 min), the pH of the supernatant solution was reduced to 5 with acetic acid (2 M), and the amylopectin fraction was precipitated[7] with iodine–potassium iodide.

Estimation of beta-amylolysis limits.—(a) By GPC. To a solution of amylopectin (5 mg) in acetate buffer (5 mL, pH 5, 20 mM) which contained 0.02% of sodium azide, beta-amylase (10 U), previously dialysed against the same solvent, was added at 0, 30, and 60 min, and the mixture was incubated at 37°C. After 90 min, the hydrolysate (200 μL) was injected onto a column (60 × 1 cm) of Fractogel TSK HW 40(S), equilibrated with the same buffer, and eluted at 0.42 mL/min at 70 psi. The maltose produced was detected by a refractive index detector (Erma Optical Works Ltd., Japan), and quantified by the phenol–H_2SO_4 method[28].

(b) By copper reduction. Amylopectin (~ 3 mg) was dissolved in 10 mL of acetate buffer (pH 5, 20 mM) and hydrolysed by adding beta-amylase (8 U at 0, 30, and 60 min) at 37°C. The reducing activity was determined by the Somogyi–Nelson method and total glucan by the phenol–H_2SO_4 method.

Preparation of beta-limit dextrins.—A solution of each amylopectin (200 mg) in Me_2SO (1 mL) was diluted with acetate buffer (50 mL, pH 5, 100 mM), incubated with beta-amylase (500 U) at 30°C, and then dialysed against the same buffer overnight. More beta-amylase (400 U) was added, the solution was dialysed against fresh acetate buffer for 24 h and then against water for 30 h, with one change of water, and boiled, and the limit dextrin was isolated by lyophilisation.

Determination of maltose and maltotriose released on debranching of the beta-limit dextrins.—A solution of each freeze-dried beta-limit dextrin (15 mg) in acetate buffer (3.0 mL, pH 5, 2 mM) that contained 0.02% of sodium azide was incubated at 37°C for 18 h with pullulanase (20 U), previously dialysed against the same buffer. An aliquot (200 μL) of the hydrolysate was injected onto a column (60 × 1 cm) of Fractogel TSK HW 40(S), and the maltose and maltotriose eluted were detected and quantified by refractive index. The total glucan was determined by the phenol–H_2SO_4 reagent.

Determination of the average chain length (CL).—A solution of the amylopectin (15 mg) in acetate buffer (10 mL, pH 5, 20 mM) was debranched at 37°C with isoamylase (20 U) for 18 h. The reducing power of the hydrolysate was determined by the Somogyi–Nelson method and the total glucan by the phenol–H_2SO_4 method.

Determination of the relative reducing power of maltosaccharides (dp 2–7).—Maltose and malto-oligosaccharides (dp 3–7, Boehringer) were determined by the Somogyi–Nelson reagent.

GPC and PC of the short chains obtained on debranching of rabbit-liver glycogen and sweet-corn phytoglycogen.—Separate solutions of rabbit-liver glycogen and sweet-corn phytoglycogen (5 mg) in acetate buffer (2 mL, pH 5, 20 mM) were each debranched by isoamylase (10 U) at 37°C for 16 h. After denaturing by heat, pullulanase (10 U) was added, each solution was incubated at 37°C for 4 h and then applied to a column (60 × 1 cm) of TSK HW40(S), and the eluate was monitored with a refractive index detector. The debranched glucans were also examined by PC on Whatman No. 1 paper (upper phase of 1-butanol–EtOH–H_2O, 4 : 1 : 5), with detection by alkaline silver nitrate[29].

REFERENCES

1 D.J. Manners, *Carbohydr. Polym.*, 11 (1989) 87–112.
2 D.J. Manners, *Carbohydr. Polym.*, 16 (1991) 37–82.
3 R.L. Whistler, J.N. BeMiller, and E.F. Paschall, *Starch: Chemistry and Technology*, Vol. 1, Academic, New York, 1984.
4 N.K. Matheson, *Phytochemistry*, 10 (1971) 3213–3219.
5 N.K. Matheson and L.A. Welsh, *Carbohydr. Res.*, 180 (1988) 301–313.

6 N.K. Matheson, *Carbohydr. Res.*, 199 (1990) 195–205.
7 S-H. Yun and N.K. Matheson, *Carbohydr. Res.*, 270 (1992) 85–101.
8 B.S. Enevoldsen and B.O. Juliano, *Cereal Chem.*, 65 (1988) 424–427.
9 S. Peat, W.J. Whelan, and G.J. Thomas, *J. Chem. Soc.*, (1956) 3025–3030.
10 G.N. Bathgate and D.J. Manners, *Biochem. J.*, 101 (1966) 3c.
11 H. Bender, R. Siebert, and A. Stadler-Szöke, *Carbohydr. Res.*, 110 (1982) 245–259.
12 N.K. Matheson, *Proc. Carbohydr. Chem. Symp., Sydney, Australia*, 1984, pp. 85–87.
13 K. Umeki and T. Yamamoto, *Agric. Biol. Chem.*, 41 (1977) 1515–1517.
14 N.W.H. Cheethan, N. Hansawek, and P. Saecou, *Carbohydr. Res.*, 215 (1991) 59–65.
15 D.J. Manners and N.K. Matheson, *Carbohydr. Res.*, 90 (1981) 99–110.
16 N. Inouchi, D.V. Glover, and H. Fuwa, *Staerke*, 39 (1987) 259–266.
17 S. Hizukuri and Y. Maehara, *Carbohydr. Res.*, 206 (1990) 145–159.
18 E. Bertoft, *Carbohydr. Res.*, 212 (1991) 245–251.
19 J.F. Robyt and W.J. Whelan, *Anal. Biochem.*, 45 (1972) 510–516.
20 D.J. Manners, J.J.M. Rowe, and K.L. Rowe, *Carbohydr. Res.*, 8 (1968) 72–81.
21 C.D. Boyer and J. Preiss, *Plant Physiol.*, 67 (1981) 1141–1145.
22 A.M. Smith, *Planta*, 175 (1988) 270–279.
23 N.K. Matheson, in D.R. Murray (Ed.), *Seed Physiology, Vol. 1*, Academic, Sydney, 1984, pp 167–208.
24 H.S. Saini and N.K. Matheson, *Phytochemistry*, 20 (1981) 641–645.
25 E. Goldsmith, S. Sprang, and R. Fletterick, *J. Mol. Biol.*, 156 (1982) 411–427.
26 Y. Konishi, Y. Ninomiya, and H. Fuwa, *Agric. Biol. Chem.*, 48 (1984) 215–217.
27 N.K. Matheson, *Phytochemistry*, 14 (1975) 2017–2021.
28 J.E. Hodge and B.T. Hofreiter, *Methods Carbohydr. Chem.*, 1 (1962) 388–389.
29 W.E. Trevelyan, D.P. Proctor, and J.S. Harrison, *Nature (London)*, 166 (1950) 444.