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α(1-4) Glucan chain disposition in models of α(1-4)(1-6) glucans: comparison with structural data for mammalian glycogen and waxy amylopectin

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Abstract

Models of the chain distribution in α(1-4)(1-6) glucans—a regularly branched and a random dendrimeric structure, as well as variations of these in which the average frequency that B chains are branched by (1-6) linked chains (*F*), and the overall, internal, core and external average chain lengths resemble those of mammalian glycogen and waxy amylopectin have been generated. In the regularly branched model, with a total of *T* chains, when *F* is between 1 and 2 (e.g. glycogen) the numbers of each type of constituent chains (A, singly and doubly branched B), are *Ta*, *Tb*(2 − *F*) and *Tb*(*F* − 1) respectively, where *a* and *b* are the fractions of A and B chains. When *F* is between 2 and 3 (e.g. amylopectin) the numbers of A, doubly branched B and triply branched B chains are *Ta*, *Tb*(3 − *F*) and *Tb*(*F* − 2). The random dendrimeric model was obtained from an algorithm with varying probabilities assigned to extension and branching of A and B chains. The data produced included the fractions of A chains, of B chains with different levels of branching and of chains in tiers, and also the relative average lengths of A chains and segments of B chains between branches. In the random dendrimeric model the average fractions of chain types are: $a, b^2, b^2a, \ldots, b^2a^{k-1}$ where the numbers of branches in B chains (term 2 onwards) are the exponent of *a* plus one.

Gaussian distributions of numbers of chains versus degree of polymerisation (in glucosyl units) (d.p.) have been constructed for each chain type from their mean d.p. and average number of chains, and these distributions convolved to give a profile of numbers of α(1-4) chains versus their d.p. For rabbit liver glycogen, conversion to normalised curves of relative weight of glucan versus d.p. and comparison with normalised chromatographic elution profiles of debranched chains indicated that the curves of all the various regularly branched models differed markedly from those of the naturally-occurring polysaccharides.

For this and waxy amylopectin a version of a random dendrimeric model was more appropriate than any of the regularly branched models and for the waxy amylopectin a significant difference in the experimental chain profile was a subsidiary peak at the d.p. corresponding to B chains with four branches in the model. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Chain distribution; Random dendrimeric structure; α(1-4)(1-6) glucans; Amylopectin; Glycogen

1. Introduction

A number of structural models of the arrangement of chains of α(1-4) linked glucan in the α(1-4) (1-6) glucans, glycogen and amylopectin, have been described and reviewed (Manners, 1989, 1991). Some models are qualitative, e.g. for glycogen (Gunja-Smith, Marshall, Mercier, Smith, & Whelan, 1970; Larner, Illingworth, Cori, & Cori, 1952; Meyer, 1943; Rani, Shibanuma, & Hizukuri, 1992) and for amylopectin (Borovsky, Smith, Whelan, French, & Kikumoto, 1979; French, 1984; Gunja-Smith et al., 1970; Hizukuri, 1986; Lee, Mercier, & Whelan, 1968; Manners & Matheson, 1981; Meyer & Bernfeld, 1940; Robin, Mercier, Charbonniere, & Guilbot, 1974). Mathematical relationships of various aspects of the chain arrangements have also been derived (Goldsmith, Sprang, &

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a, fraction of A chains; *b*, fraction of B chains; B*a*, B chains with only A chains attached; B*b*, B chains with B chains and possibly A chains attached; B_A, probability of branching an A chain; B_B, probability of branching a B chain; B_n, B chain with *n* branches; CCL, average core chain length (in glucosyl units) ; CL, average chain length of A + B chains; CL_A, average chain length of A chains; CL_B, average chain length of B chains; CL_{B_n}, average chain length of B chains with *n* branches; d.p., degree of polymerisation (in glucosyl units) ; DP, mean degree of polymerisation of a particular type of chain, such as A, B_1; E_A, probability of extension of an A chain; E_B, probability of extension of a B chain; ECL, average external chain length; ECL_B, average chain length of segments of B chains external to the outermost branch point; *F*, average frequency that B chains are branched by (1-6) linked chains; ICL, average internal chain length; IEC, ion-exchange chromatography; L_B, average length of B segments (both internal and external); *m*, mean number of tiers; *N*, value of the highest tier number; PAD, pulsed amperometric detection; SEC, size exclusion chromatography; *T*, total number of chains; *w*, range of d.p. at half height of the distribution of a population of a particular type of chain.

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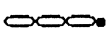
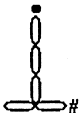
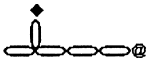
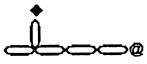
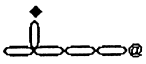
Table 1
Configurational requirements for either extension or branching of randomly branched dendrimer

PROGRAM	Pre-event configuration	Pre-event configuration status (CS)	Event	Post-event configuration	Post-event configuration status (CS)
1		● CS = 1	Extension (of an A chain)		● CS = 1
1&2		● CS = 1	Branching (of an A chain)		◆ CS = 2 # CS = 3
1&2		◆ CS = 2 # CS = 3	Extension (of an A chain)		■ CS = 4 # CS = 3
1&2		◆ CS = 2 # CS = 3	Extension (of a B chain)		◆ CS = 2 □ CS = 5
1&2		■ CS = 4 # CS = 3	Extension (of an A chain)		● CS = 1 # CS = 3
1&2		◆ CS = 2 □ CS = 5	Extension (of a B chain)		◆ CS = 2 @ CS = 6
1		◆ CS = 2 @ CS = 6	Extension (of a B chain)		◆ CS = 2 @ CS = 6
1&2		◆ CS = 2 @ CS = 6	Branching (of a B chain)		◆ CS = 2 # CS = 3

Fletterick, 1982; Gunja-Smith et al., 1970; Matheson, 1996; Stetten & Katzen, 1961; Thurn & Burchard, 1985; Yun & Matheson, 1993).

Several numerical values of aspects of the structural disposition of the α(1-4) glucan chains that form the biodendrimers can be determined experimentally—the fractions of A and B chains (*a* and *b*) and hence the A:B chain ratio (Bathgate & Manners, 1966; Enevoldsen & Juliano, 1988; Manners, 1985, 1989, 1991; Peat, Whelan, & Thomas, 1956; Whelan, 1971; Yun & Matheson, 1993); the average degree of polymerisation of α(1-4) glucan chains (CL); the average external chain length (ECL) and internal chain length (ICL) (Manners, 1985, 1989, 1991; Whelan, 1971); the average core chain length (CCL) and average frequency that B chains are branched by (1-6) linked chains (*F*) (Yun & Matheson, 1993; Matheson, 1996) and the distribution of chain lengths of the α(1-4) chains (Akai, Yokobayashi, Misaki, & Harada, 1971; Cheetham, Hansawek, & Saecou, 1991; Hizukuri, 1986; Lee et al., 1968; MacGregor & Morgan, 1984; Manners, 1989, 1991; O'Shea & Morell, 1996; Palmer, Macaskie, & Grewal, 1983a). Differences are found between glycogen and amylopectin for these values. The A:B chain ratios, the various average chain lengths and the *F* values are lower for glycogens than for amylopectins, and the distribution of α(1-4) chain lengths varies. Glycogens have a unimodal, skew distribution of α(1-4) chain lengths, whereas amylopectins have bi- or polymodal patterns. For some amylopectins from normal starches their profiles of numbers of α(1-4) chains versus d.p. have been deconvolved into sets of Guassian (Ong, Jumel, Tokarczuk, Blanshard, & Harding, 1994) or Poisson distributions (Erlander, 1998a). Amylopectin solutions are more viscous than those of glycogens and M_W of the former is higher. Glycogens differ among themselves. Mammalian glycogens have higher values for the various types of average chain lengths than those from invertebrate sources (Manners, 1991). Amylopectins also differ between genotypes and between species. In maize, waxy genotypes have the lowest values for the various average chain lengths, which increase for normal starch and then increase again

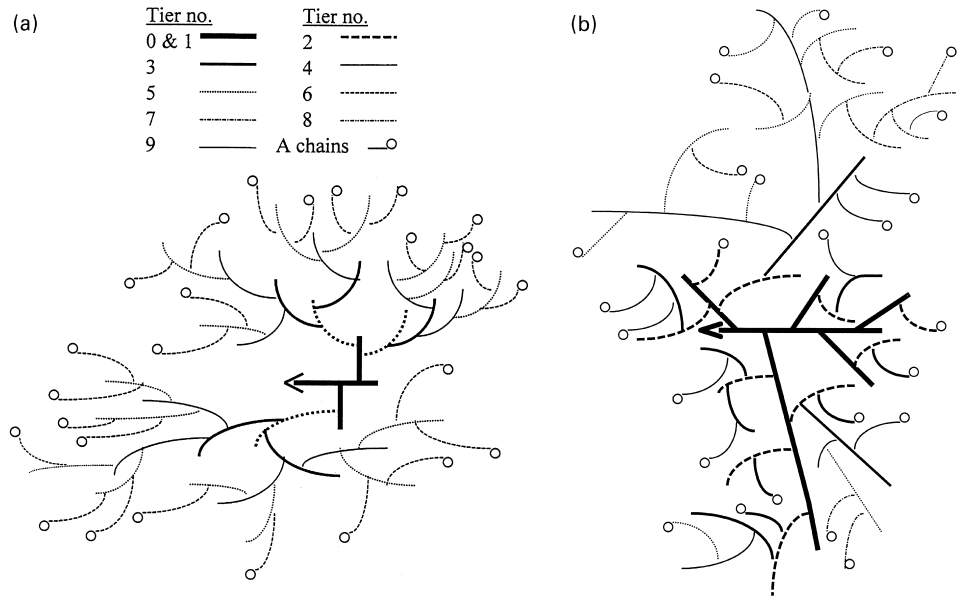


Fig. 1. a: An illustration of an arrangement of α(1-4) chains in the regularly branched model ($F = 1.72$, $T = 61$); b: an illustration of an arrangement of α(1-4) chains in the random dendrimeric model.

for the amylose-extender genotypes (Matheson, 1996). However, the glycogens group together in one general type of structure and the amylopectins in another. Based on enzymic degradation, chemical studies and physicochemical properties, the models of glycogen usually proposed are symmetrical, relatively regular, spheroidal polymers, whereas those for amylopectin present more elongated, ellipsoidal structures, with clusters of dendritic segments joined by longer B chains. Both regular and non-regular branching patterns have been depicted.

In the following discussion the α(1-4) glucan chain arrangements of two basic models, regularly branched and random dendrimeric, with variations of these, are described. Calculated α(1-4) glucan chain profiles of models having the *a* values and average chain lengths of mammalian glycogen and amylopectin are compared with experimental profiles found for these polymers.

2. Methods

2.1. Algorithm for the generation of a random dendrimeric structure

The dendrimeric structure evolves by a combination of two fundamental events. The first possible event is the addition of a link to a precursor chain causing extension of the chain. The second event is branching, when a precursor chain branches at a defined point in the chain. Two programs have been developed. Depending on the program chosen, extension or branching will occur only on a chain when a configurational requirement (indicated by its configurational status) of the precursor chain is satisfied. A schematic representation of these configurational requirements is shown in Table 1.

In program 1, chains may extend further than three links before a branching event occurs, whereas in program 2 branching is the only event that may occur to a sequence of three unbranched links. The initial structure with which the program commences is a linear three-link chain, of which only one end can undergo extension or branching. Therefore in program 1 it may either extend or branch whereas in program 2 the first event is a branching one.

At any instant during the evolution of the structure there are chains which have not branched (termed A chains) and those that have branched (termed B chains). Branching of an A chain creates a new A chain and converts the parent A chain to a B chain. Branching of a B chain again creates a new A chain and introduces a further branch into the parent B chain. In these programs, branching has been defined as occurring by the re-arrangement of the terminal link to a point at the junction point of the preceding two links (i.e. branching is always intra-chain). This stipulation leads to a possible minimum of one link between branch points.

The programs permit the initial assignment of relative probabilities to the extension and branching events of either A or B chains. Either program, on accessing a random number (between 0 and 1), determines the event to occur and the chain to be modified. The configurational status of the parent and any daughter chain is altered according to the protocol outlined in Table 1. After the event the relative probabilities are adjusted to account for a change in the overall number of configurations. The program accesses a new random number for each event and terminates when a pre-assigned number of chains have been generated. It may be repeated a number of times with the same starting

Table 2
Types of chains in the regularly branched (Fig. 1a) and random dendrimeric (Fig. 1b) models with $T = 61$, $F = 1.74$

	Regularly branched model			Random dendrimeric model		
Tier number	Number of B chains in tier	Number of A chains in tier	$\sum$	Number of B chains in tier	Number of A chains in tier	$\sum$
0	1	0	1	1	0	1
1	2	0	2	5	0	5
2	3	0	3	8	2	10
3	5	0	5	7	4	11
4	9	0	9	4	8	12
5	15	0	15	5	5	10
6	0	26	26	3	2	5
7	0			1	3	4
8	0			1	1	2
9	0			0	1	1
Chain type	Number			Number		
B_1	11			22		
B_2	24			7		
B_3				3		
B_4				1		
B_5				2		
B_a[a]	15			20		
B_b[b]	20			15		

[a] B chains with only A chains attached.
[b] B chains with B, and possibly, A chains attached.

parameters, thereby producing means and standard deviations for the data obtained. On completion the following information is presented:

1. the fraction of A chains;
2. the number of chains with specific chain lengths;
3. the number of B chains with a specific number of branch points;
4. the number of chains belonging to a specific tier level;
5. the number of links incorporated in the structure in order to achieve a pre-set number of chains under a specified set of probabilities;
6. the average A chain length;
7. the average number of links between branch points of B chains.

2.2. Construction of convolved curves of weight of glucan in α(1-4) chains versus d.p.

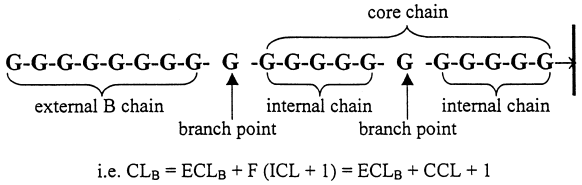
Gaussian distributions for each chain type and their convolved curves were constructed with MicroCal Origin® software.

3. Results and discussion

3.1. Regularly branched models

The basic regularly branched model has a structure in which the total number of branch chains linked to all the B chains in a tier is equal to $F \times$ no. of B chains in that tier (or as close as possible for low-numbered tiers). If F is less than 2 (as is found for glycogens) then the fractional amounts of the different types of chains are described by the terms a, $b(2 - F)$, $b(F - 1)$, where a is the fraction of A chains, $b(2 - F)$ of singly branched B chains and $b(F - 1)$ of doubly branched B chains: there are no other types of chains. The numbers of chains in tiers are defined by the sequence $F^0, F^1, F^2, \ldots, F^N$, where the superscript is the number of a tier, N the total number of tiers and $F = 1/(1 - a)$ (Yun & Matheson, 1993). For a polymer with a total of T chains the numbers of each type of chain are Ta, $Tb(2 - F)$ and $Tb(F - 1)$. If the F value lies between 2 and 3 (as in amylopectin) the numbers of the different types of chains are Ta, 0, $Tb(3 - F)$ and $Tb(F - 2)$ where the first term is the number of A chains: there are no B chains with one branch and the next two terms give the numbers of chains with two and three branches. Fig. 1a gives a two-dimensional (2D) representation of this model in which $T = 61$ and $F = 1.74$. The numbers of the various types of chains are shown in Table 2. Chains A have no other chains linked to them via the primary (O-6) hydroxyls of their constituent glucosyl units but B chains do. Ba chains have only A chains as branches, whereas Bb chains have either only B chains or both B and A chains attached (Hizukuri & Maehara, 1990).

Chains A are wholly exterior to the outermost branch points, whereas B chains consist of a section exterior to the outermost branch point plus the internal chain segments and one glucosyl branching unit per internal segment.



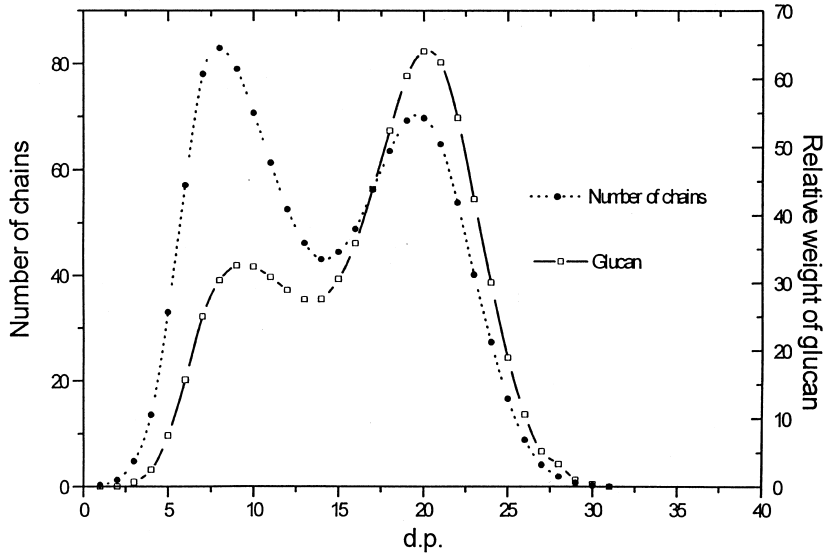


Fig. 2. Number of α(1-4) chains or relative weight of glucan versus d.p. in the regularly branched model of glycogen ($a = 0.42$, $T = 930$, CL = 14, ICL = 5, $ECL_B = CL_A = ECL = 8$: A chains convolved as 91 of DP 6.8 with $w = 3$ and 300 at DP 9 with $w = 5$; 151 B_1 and 388 B_2 chains with $w = 6$).

ECL_B and CL_A cannot be measured separately, only as a mean (ECL) which equals $b ECL_B + a CL_A$. From F, ECL_B, CL_A, ICL, a and T the numbers of types of α(1-4) glucan chains versus their mean degree of polymerisation (DP) can be calculated.

3.2. Comparison of regularly branched and modified regularly branched models with two mammalian glycogens (rabbit and bovine liver)

Rabbit liver glycogen has been found to have an a value of about 0.4–0.45, CL 13–16, ICL 3–5, ECL 8–9 and CCL 10. For a regularly branched polymer with $a = 0.42$ ($F = 1.72$), CL = 14, in which $T = 930$ (i.e. d.p 13 020, M_W 2.1×10^6 with 11 tiers and 1, 2, 3, 5, 9, 15, 26, 45, 77, 132, 227 and 390 chains to the nearest integer in tiers 0–11, total 930) the number of A chains is 391, B_1 chains 151 and B_2 chains 388. The subscript to B defines the number of branches in a B chain. Increasing the M_W increases the numbers of each type of chain but the proportions of these remain the same.

There are variations of the regularly branched model that have the same numbers of chain types versus DP. These must have all chains only singly or doubly branched and maintain the F value overall. In the *first variation* A chains on all or some of the doubly branched B chains of the penultimate tier are re-positioned on B chains in tiers lower than the penultimate that are only branched by one B chain. There is a limit to the number of movable A chains.

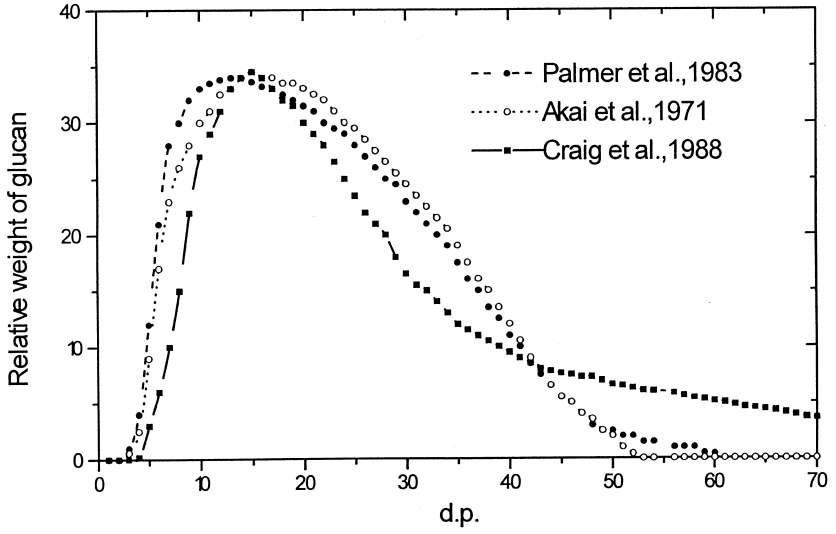


Fig. 3. Weight of glucan versus d.p. of α(1-4) chains of rabbit liver glycogen.

For F values ≤ 2 the number of available B_1 chains is

$$(2 - F) \times \sum_{N=1}^{N-2} F^N.$$

For glycogen (F, 1.72; T, 930) this number is 88.

In the *second variation* the proportions of doubly and singly branched B chains in each tier from 1 to $(N - 1)$ differ from the F value but the overall F value is maintained. The degree of movement may be from 1 to a limit set by the F value. The two variations may also be combined in various proportions.

Allowing for a distribution of chain lengths about the DP of each type of chain, distributions of number of chains versus d.p. for each type of chain can be calculated from the DP values, the numbers of types of chains and standard deviations. These can then be convolved to give a profile of numbers of α(1-4) chains versus d.p. for the model. Skew distributions for a chain type can be constructed by splitting the DP and number of chains into fractions. The relative weight of glucan is then obtained by multiplying the number of chains at each d.p. by the d.p. and a profile of weight of glucan versus d.p. (after normalising) can be compared with experimental elution profiles of debranched polymers (Palmer et al., 1983a,b).

Fractionation of debranched chains of mammalian glycogen and waxy amylopectin has been effected by three types of methods; size exclusion chromatography (SEC) on various matrices in alkaline, neutral ionic or non-ionic aqueous solutions; ion exchange chromatography (IEC) in alkali; and electrophoresis of fluorescent-labelled chains. Diffusion of chains occurs on the column in the first, but in the second and third baseline separation of chain lengths is achieved. However, with a debranched waxy rice amylopectin the elution profiles by SEC (Hizukuri, 1986) and by IEC with pulsed amperometric detection (PAD) of joined peak values (Hanashiro, Abe, & Hizukuri, 1996) have given reasonably similar profiles (see Fig. 6).

Two characteristics of experimental elution profiles suggest that the range of d.p. values of a single chain type (e.g. A, B_1) is not excessively wide. The peak of weight of glucan in glycogens with a 0.42 occurs at the DP of B_1 chains, whereas in waxy amylopectins ($a = 0.56$) it is at the DP of A chains (see Section 3.4 and Fig. 3): also, in the latter there is a separation of peaks (or a point of inflection) between the DP of A and B_1 chains (see Fig. 6). A mechanism of enzymic branching with an optimal chain length for scission would have the effect of limiting the variation in lengths within types of B chains with levels of branching higher than one. The d.p. of A chains or external segments of B chains that varied much from the average length would revert to nearer the average on the next branching. In a distribution the range of d.p. values at half height is w, so that 95% of chains lie within d.p. values of $2w$ monomer units.

In plots of numbers of chains versus d.p. for the regularly branched model, constructed by convolving Gaussian distributions, initial calculations were made with $ECL_B = CL_A =$ ECL. A w value of 6 was used; that is, 95% of the chains lie within a range of 12 glucosyl units. To avoid the appearance of a significant number of chains of d.p. < 4 the population of A chains was produced as a skew distribution by combining two curves which still had 95% of chains within the d.p. range of 12. The convolved distribution of numbers of chains versus d.p., and of relative weight of glucan versus d.p. derived from it, are shown in Fig. 2. In Fig. 3, three redrawn experimental elution profiles of rabbit liver glycogen, in which weight of glucan (normalised to the same total weight as in Fig. 2) versus d.p. are plotted. One of the profiles was obtained by SEC on Bio-Gel® P-10 at neutral pH, with d.p. and glucan contents estimated by Nelson–Somogyi copper reduction before and after enzymic depolymerisation (Palmer et al., 1983a) and another is the result of SEC on Sephadex® G-75 in 0.05 M NaOH, with estimation of d.p. by production of formaldehyde from periodate oxidation of $NaBH_4$-reduced glucan (Akai et al., 1971). The third profile from SEC on Sephadex® G-75 in water (Craig, McDonald, Manners, & Stark, 1988) gave a curve of similar shape with a single maximum at d.p. 14 (max 34.5) but a higher range of chain lengths (up to > 100). The profile of α(1-4) chains in the model is quite different from any of these. The model has two maxima for weight of glucan (at d.p. 9 and 20) compared to one peak in the experimental profiles (at d.p. 13–15); the glucan weights at these maxima are 41 and 173 compared with 34; and the highest d.p. is 24 in contrast to at least 52 experimentally. With w at values from 3 to 8 the curves of weight of glucan versus d.p. for the regularly branched model were all quite different from the experimental plot: at $w = 8$ the curve has a significant number of chains to the negative side of the ordinate axis.

Since $ECL = aCL_A + bECL_B$ and a small number of chains with a d.p. of less than 5 is released on debranching of rabbit liver glycogen, the maximum possible length for ECL_B is $(8 - 0.42 \times 5)/0.58 = 10$, and, since there is a significant decrease in the d.p. of longer chains on β-amylolysis, ECL_B is not extremely short. Also, the proportions of A and B chains being not far from a half suggests that ECL_B and CL_A would not differ greatly. When ECL_B was varied between 6 and 10, while maintaining ECL at 8, the calculated curves of weight of glucan versus d.p. still had two peaks and were quite different from the experimental profiles. When ECL_B was 6 the peak heights were 69 and 77 at d.p. 12 and 18, and when ECL_B was 10 the heights were 29 and 89 at d.p. 7 and 22.

Another variation of this regularly branched model is the *modified* regularly branched model with regular branching of B chains in each tier at the F value but increased chain lengths of some chains, giving a B chain profile containing appropriate numbers of chains longer than d.p. 20 (B_2 chains in the regularly branched model) so resembling the experimental profile above d.p. 23. Since CL, ICL, CCL, ECL and F are all defined and must be maintained, extension of some

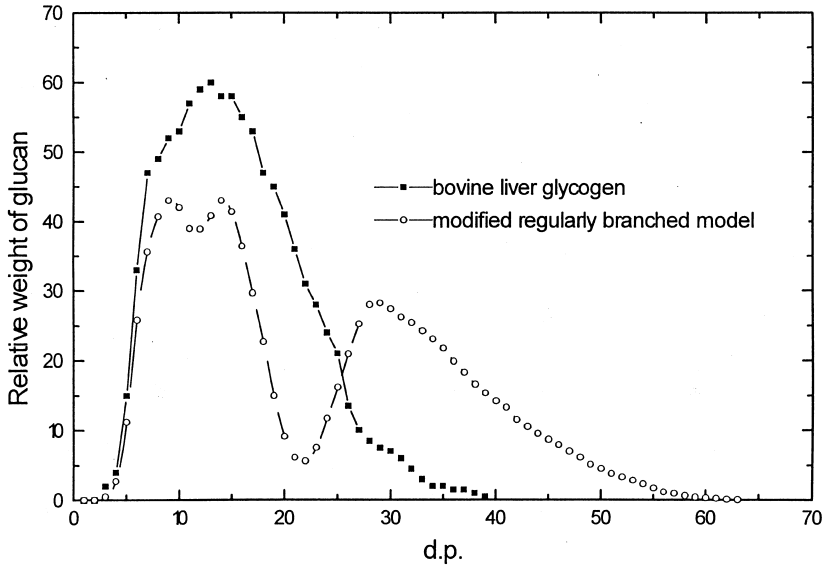


Fig. 4. Weight of glucan versus d.p. of bovine liver glycogen chains (O'Shea & Morell, 1996) and of modified regularly branched model of glycogen ($a = 0.42$, $T = 930$, CL = 14, ECL = 8, ICL = 1, $ECL_B = 9.5$, $CL_A = 6$, A chains convolved as 71 of DP 5 with $w = 2$ and 320 of DP 7 with $w = 4$; 151 B_1 chains of DP 11.5; 190, 93, 56, 29, 14, 5 and 1 B_2 chains of DP 13.5, 26.5, 32.5, 38.5, 44.5, 50.5 and 56.5, all with $w = 6$).

B chains will involve reduction of the internal chain lengths of other B chains or alteration of either CL_A or ECL_B within an overall constant ECL of 8, or of both. For a constant F the numbers of B_1 and B_2 chains must not vary. Estimates of the number of chains with higher DP were made by measuring sectional areas with abscissa lengths of d.p. 6 in the experimental elution profile of weight of glucan obtained by Palmer et al. (1983a). These were converted to proportions of number of chains by multiplying by the DP of the segment. For sections of d.p. 24–29, 30–35, 36–41, 42–47, 48–53, 54–59 the numbers of chains were estimated ($T = 930$) as 93, 56, 29, 14, 5 and 1—a total of 198. With an ECL of 8, chains of d.p. > 23 are probably too long to be A chains. Since $CL = aCL_A + bCL_B$ and few chains with a d.p. of less than five are released on debranching rabbit liver glycogen, the maximum possible value for CL_B is 20.5. Since $ECL = aCL_A + bECL_B$, if CL_A is 6 (a reasonable minimum mean if its minimum chain length is 4–5) then ECL_B would be 9.5 and CL_B 19.8. Extension of 198 B_2 chains of the regularly branched model leaves $388 - 198 = 190$ unextended B_2 chains plus the original B_1 chains. To maintain the observed ICL and CCL these must be shortened. The required average internal chain length of the shortened chains (*ICL*) can be calculated from:

$$CL_B = \{B_1[ECL_B + (ICL + 1)] + \text{shortened } B_2\,[ECL_B + 2(ICL + 1)] + \sum(\text{extended } B_2 \times \text{chain length})\}\ /T_B,$$

which gives $ICL = 1$.

If CL_A were 5, then CL_B would be 20.5, ECL_B 10.2 and *ICL* 1.3, and if CL_A were 7 the values would be 19, 8.7 and 0.8: with CL_A and ECL_B maintained at 8, CL_B would be 18 and *ICL* 0.5. A plot of weight of glucan versus d.p. for this *modified* regularly branched model with $ICL = 1$ is shown in Fig. 4. It still differs markedly from the experimental profiles of rabbit liver glycogen (Fig. 3). This model also requires that many of the internal chains (and hence core chains) be very short.

Bovine liver glycogen has been debranched, and, after fluorescent labelling of the α(1-4) chains, these were separated by electrophoresis and the amounts estimated from the level of labelling and the position of elution (O'Shea & Morell, 1996). Re-plotting their data as weight of glucan versus d.p. and normalizing to the same area as the regularly branched model gave the curve shown in Fig. 4, different from that of the regularly branched and *modified* regularly branched models but also differing from those of rabbit liver glycogen (Fig. 3)—bovine liver glycogen had a lower maximum d.p. (40 compared to > 53), and a higher maximum of glucan content at the peak value (60 compared to 34), but the d.p. values at which the maxima occurred were similar (13–14). Possible reasons for the difference in the experimental profiles include different methods of chromatography and analysis. However, glycogen is in a metabolically mobile state and they may have been sampled at different physiological states. The metabolism of glycogen also involves debranching enzyme.

3.3. Comparison of the regularly branched and modified regularly branched models with waxy amylopectins

CL values of 18–20, ECL values of 12–13 and ICL values of 5–6 have been found for waxy rice starch. With $F = 2.27$ ($a = 0.56$), CL = 19 and $T = 1260$ (i.e. d.p. 23,940, M_W 3.9×10^6 with 8 tiers and 1, 2, 5, 12, 27, 60, 137, 311, 705 chains in tiers 0–8 i.e. a total of 1260) the number of A chains is 705 (DP = 12), B_1 chains 0, B_2 chains 405 (DP = 26) and B_3 chains 150 (DP = 33). Two plots of weight of glucan versus d.p. with w values of 6 and 7 are

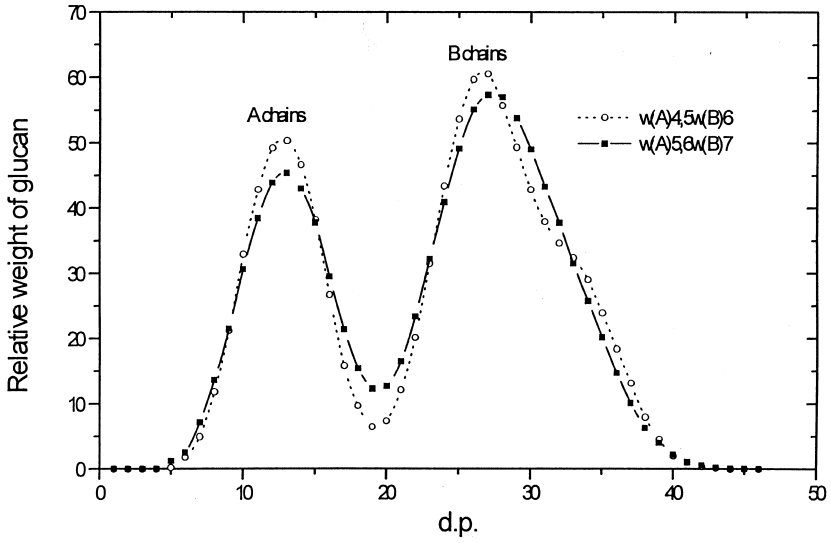


Fig. 5. Weight of glucan versus d.p. of α(1-4) chains in the regularly branched model of amylopectin ($a = 0.56$, $T = 1260$, CL = 19, ICL = 6, $ECL_B = CL_A = ECL = 12$; for w(A)4,5w(B) 6, 200 A chains are of DP10 and $w = 4$ and 505 of DP13 and $w = 5$; for w(A)5,6w(B) 7, 200 A chains are of $w = 5$ and 505 of $w = 6$).

shown in Fig. 5. It can be compared with the very different distribution of α(1-4) chains of debranched waxy rice starch (Fig. 6) derived from the data of Hizukuri (1986), obtained by SEC on Fractogel® with detection by differential refractometry and also by IEC-PAD (Hanashiro et al., 1996) and of waxy barley by SEC on Bio-gel P-6 with assay by orcinol–H_2SO_4 (MacGregor & Morgan, 1984) (Fig. 6). These amylopectins and that from waxy maize (Yun & Matheson, 1992) have given similar types of profiles, with further peaks (or inflection points) at d.p. values higher than where the maximum of the curve occurred. The maximum peak for these waxy starches is at d.p. 13, corresponding to the DP of A chains if CL_A and ECL_B are similar, whereas in glycogen the peak is near the DP value for B_1 chains–this is a consequence of the lower a value of glycogen than of amylopectin (see Section 3.4). If ECL_B was varied between 10 and 14—while maintaining ECL at 12—calculated curves of relative weight of glucan versus d.p. still consisted of two peaks, differing from the experimental curves. With ECL_B at 10, peak heights of 58 and 56 occurred at d.p. 15 and 25 and a point of inflection was found near 30. With ECL_B at 14 heights were 43 and 61 at d.p. 11 and 29, with a point of inflection at d.p. 33. The distributions were always quite different from those of native polymers.

Forming a profile of numbers and range of chains by shortening some B_2 and B_3 chains to the length of B_1 chains (maintaining the numbers of branches at 2 and 3) and lengthening some B_3 chains to the lengths of B_4–B_8 chains—while keeping ECL at 12—to give similar numbers of chains versus d.p. to the experimental profile obtained from IEC-PAD by Hanashiro et al. (1996), produces a polymer with an *ICL* of 4.5. To maintain an

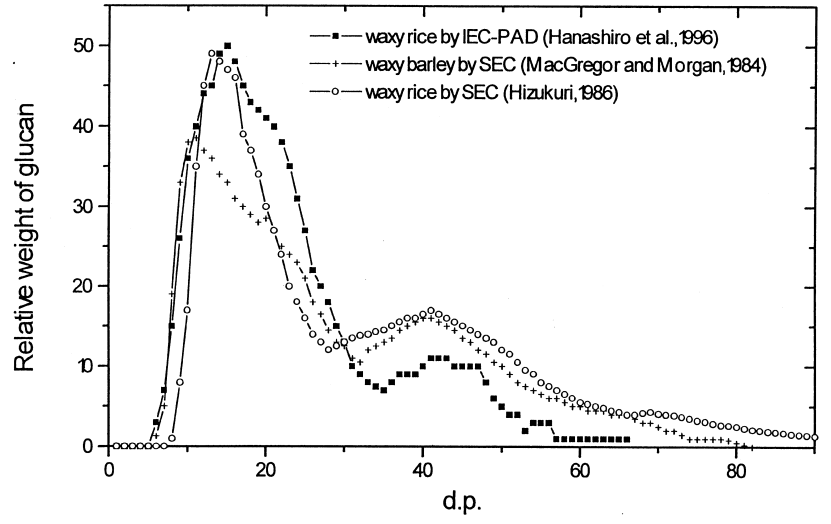


Fig. 6. Elution profiles of debranched chains of waxy rice and barley amylopectins.

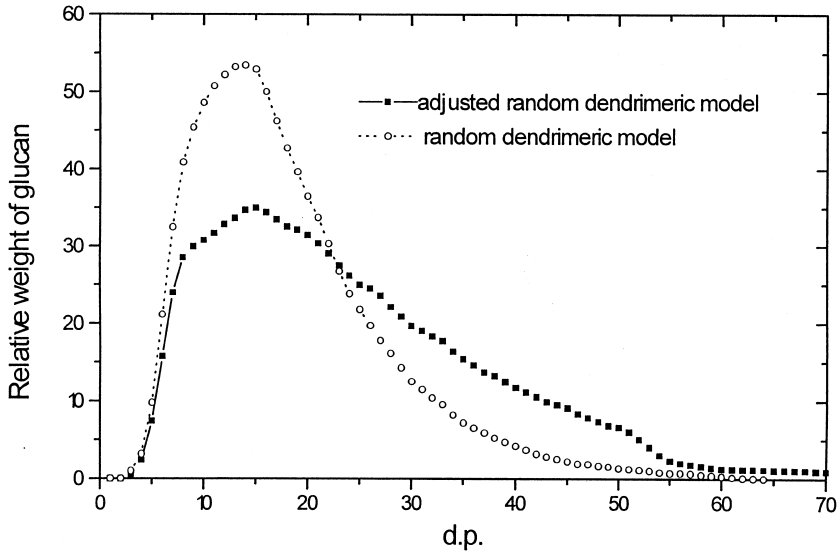


Fig. 7. Weight of glucan versus d.p. of α(1-4) chains in the random dendrimeric and the *adjusted* random dendrimeric models of glycogen ($a = 0.42$, $T = 930$, CL = 14, ICL = 5,$ECL_B = CL_A$ = ECL = 8; A chains were convolved as 91 of DP 6.8 with $w = 3$ and 300 of DP 9 with $w = 5$; B chains with $w = 6$).

Table 3
Types of transfer from a chain

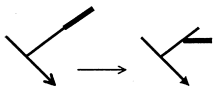
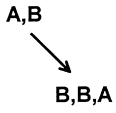
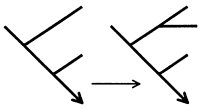
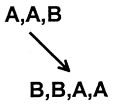
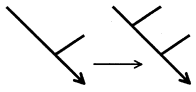
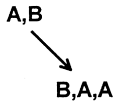
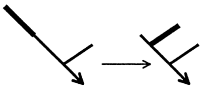
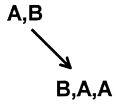
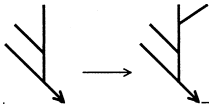
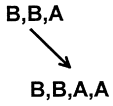
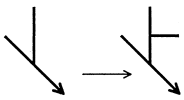
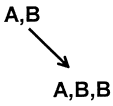
	Type of chain transferred		Reaction	Change in chain type	Net change in chains
(i)	A	intra			+ B
(ii)	A	inter to A			+ B
(iii)	A	inter to B			+ A
(iv)	B	intra			+ A
(v)	B	inter to B			+ A
(vi)	B	inter to A			+ B

Table 4
Numbers and percent of total glucan of the various types of chains in the random dendrimeric model with different a values and $T = 1000$

Type of chain	a value									
	0.30		0.40		0.50		0.60		0.70	
	No. of chains	% of glucan[a]	No. of chains	% of glucan[a]	No. of chains	% of glucan	No. of chains	% of glucan	No. of chains	% of glucan[a]
A	300	17.2	400	22.9	500	28.6	600	34.4	700	40.2
B_1	490 } 637	49.2 } 70.3	360 } 504	36.0 } 56.6	250 } 375	25.1 } 43.0	160 } 256	16.0 } 29.7	90 } 15.3	9.0 } 18.0
B_2	147	21.1	144	20.6	125	17.9	96	13.7	63	9.0
B_3	44	8.2	58	10.8	63	11.7	58	10.8	44	8.2
B_4	13 } 2	3.0 } 12.6	23 } 94	5.3 } 19.8	31 } 118	7.1 } 25.7	35 } 126	8.0 } 28.3	31 } 112	7.1 } 26.0
B_5	4	1.1	9	2.4	16	4.4	21	5.7	22	6.0
B_6	1	0.3	4	1.3	8	2.5	12	3.8	15	4.7
B_7			1	0.4	4	1.4	7	2.5	11	3.9
B_8			1	0.4	2	0.8	4	1.6	7	2.8
B_9					1	0.4	2	1.3	5	2.2
B_{10}							1	1.0	4	2.0
B_{11}							1	0.6	3	1.6
B_{12}									2	1.1
B_{13}									1	0.6
B_{14}									1	0.7
B_{15}									1	0.7

[a] For calculation of % of total glucan ECL = ECL_B = 8 and ICL = 5.

ICL of 6 an average of 3.4 glucosyl units would need to be transferred from external B chains to core chains. Then, to keep an ECL of 12, CL_A would become 15 and the average lengths of B_1 and B_2 chains 16 and 23 respectively, giving a different profile of relative weight of glucan versus d.p. to that found experimentally.

Hizukuri and Maehara (1990) estimated the proportion of Ba chains to Bb in an amylopectin β-limit dextrin by an enzymic method that depends on the slower debranching of malto-biosyl and -triosyl stubs than of longer chains. In the regularly branched model with a completed outer tier, all the chains in the outermost tier are A chains, those in the next lower tier Ba chains and the remainder in other tiers Bb chains, hence

$$\frac{\mathrm{Ba}}{\mathrm{Bb}} = \frac{F^{N-1}}{\sum_{N=0}^{N-2} F^N} = \frac{(1-F)F^{N-1}}{1-F^{N-1}} = \frac{(F-1)}{1-F^{1-N}}$$

which tends to $(F - 1)$ as N increases.

For an amylopectin with $a = 0.56$ ($F = 2.27$) when N is 9 $Ba/Bb = 1.272$ and when N is 10 it is 1.271, whereas the experimental ratio for normal wheat amylopectin was higher (1.5) indicating that, if the regularly branched model were applicable, it would need to be a variation with A chains on B chains in tiers other than the penultimate.

The data indicate that neither glycogen nor amylopectin can be adequately represented by a regularly branched model or any of its variations.

3.4. Random dendrimeric models

The arrangement and numbers of the various chain types in a random 2D dendrimeric model are described in Fig. 1b and Table 2. It has a structure with the same number of chains and F value as the regularly branched model of Fig. 1a and Table 2. In contrast, the number of tiers is higher and the number of chains in each tier, while initially increasing, subsequently decreases. Also, A chains are spread throughout the tiers and not confined to the outermost tier, and B chains have 1—5 chains linked to them instead of just 1 or 2.

The process of the formation of a dendrimeric structure can be divided into three operations, the extension of an α(1-4) gluco-oligosaccharyl chain, scission when the chain reaches an appropriate length and the joining of the reducing end of the scission product to a 6-OH. The chain undergoing branching can be seen as made up of three malto-oligosaccharyl units (links in the algorithm—Table 1); an initially transferred stub and an extension required before branching enzyme can operate. The latter is divided into the part transferred and that remaining—the former becoming a new stub (Fig. 9). Considering the splitting of a single chain, if an A chain branches via intra-chain transfer (this transfers a part of this A chain to itself) a new B chain and a replacement A chain are formed (Table 3; (i)). If inter-chain transfer occurs from one A chain to another A chain, a new B chain and a replacement A chain are produced (Table 3; (ii)). If there is inter-chain transfer from an A chain to a B chain a new A chain is created (Table 3; (iii)). If a B chain transfers intra (Table 3; (iv)) or inter to a B chain (Table 3; (v)) a new A chain results. If an enzyme mechanism of branching involving double helix formation is operating (Borovsky et al., 1979) (Fig. 9) then transfer will always create an additional chain of the opposite type to the chain being branched (A → B, A and B → A, B—pseudo intra-chain transfer).

The random dendrimeric model was generated by an algorithm involving intra-chain transfer (or pseudo intra-chain transfer). In this a restriction that at least three end-to-end links must form before branching can occur was used. It is a procedure to obtain a randomly constructed model, not necessarily a model for enzyme action, although it may reflect some aspects of the process.

The distribution of average fractions of A and B chains with differing degrees of branching in a population of molecules was always defined by the a value, whatever the set of E_A, E_B, B_A and B_B values or the program applied, and fitted the sequence:

$$a, b(b, ba, ba^2, ba^3, \ldots, ba^{k-1})$$

in which, because $b = 1 - a$, and $1 > b > 0$, the sequence within the brackets converges and, as k increases, the sum of the series approaches a limit of one. The average fraction of A chains is defined as a. The next term is the average fraction of singly branched B chains. This class of chains is produced by extension and intra-chain branching of an A chain. The third term is the average fraction of doubly-branched B chains—a doubly-branched B chain is produced by extending and branching a B chain after initial extension and branching of the A chain. B chains branched k times are produced by initially extending and branching an A chain followed by $(k - 1)$ extensions and branchings of the B chain.

In a polymer with a total of T chains the distribution of average numbers of chains is:

$$T = Ta, Tb^2, Tb^2a, Tb^2a^2, Tb^2a^3, \ldots, Tb^2a^{k-1}$$

The value of $k - 1$ (the limit of effective B chain branching) is conveniently defined as when $Tb^2a^{k-1} < 0.5$. In Table 4 the numbers of chains for a values of 0.3, 0.4, 0.5, 0.6, and 0.7 and the percent of glucan in each chain type for a polymer with ECL = ECL_B = 8 and ICL = 5 are given for polymers with 1000 chains. One aspect of this model is that for a particular value of T the extent of multiply-branched B chains increases as a increases. Another is that the decrease in the sum of B_1 plus B_2 chains that occurs as a increases is accompanied by an increase in the sum of $B_3 + B_4 + B_5 + B_6$ chains up to $a = 0.6$. This increase is paralleled by the percentage of the sum of glucan in $B_3 +$

$B_4 + B_5 + B_6$. This effect is a consequence of the series including both a and b, when $b = (1 - a)$. At high values of a the peak of the profile of weight of glucan versus d.p. occurs at the DP of A chains. However, as a decreases, depending on CL_A and CL_{B_1}, the location of the peak moves to the DP of B_1. Since the a value at which the weight of glucan in A chains is equal to that in B_1 chains occurs when $aCL_A = b^2CL_{B_1}$ the a value below which the weight of glucan in B_1 chains is greater than the weight of glucan in A chains is:

$$\left[\frac{2CL_{B_1}}{CL_A} + 1 - \sqrt{1 + \frac{4CL_{B_1}}{CL_A}}\right] \div \frac{2CL_{B_1}}{CL_A}.$$

Thus a polymer with $a = 0.42$, $CL_A = 8$ and $CL_{B_1} = 14$ has its elution peak at the DP of B_1 chains, since the changeover point for when the weight of B_1 chains is greater than A chains occurs when a is 0.48. Mammalian glycogens ($a = 0.42$) have their elution peak at the d.p. of B_1 chains. On the other hand, a polymer with $a = 0.56$ $CL_A = 12$ and $CL_B = 19$ has its peak at the d.p. of A chains since the changeover is at 0.46. Waxy rice amylopectin ($a = 0.56$) has its peak at the DP of A chains.

The range of types of B chains is only slightly dependent on M_W. Doubling the M_W produces one B chain with an extra branch. For particular values of a and T the range of chain lengths is determined by ECL and ICL. For models derived with the characteristics of maize amylopectins and $T = 1260$, the maximum chain lengths are 89 for waxy (ECL 12, ICL 6) 102 for normal (ECL 14, ICL 7) and 143 for amylose extender (ECL 22, ICL 10).

3.5. Comparison of the random dendrimeric and adjusted random dendrimeric models with mammalian glycogens

The distributions constructed from the DP and numbers of types of chains of the random dendrimeric model with the parameters of rabbit liver glycogen (program 1) were convolved with a skew distribution for A chains which avoided the generation of significant numbers of chains with a d.p. of less than 4. With w less than 6 the curves had peaks at the DP of each type of chain, and this is not observed in experimental elution profiles of debranched glycogen. Construction of the curve of weight of glucan versus d.p. (Fig. 7) and comparison with the normalised elution profile of debranched rabbit liver glycogen (Fig. 3; Palmer et al., 1983a) shows that they both have a single maximum at a similar d.p. (13–14) and the same range of chain lengths (from 4–5 to 56–60). However, the amount of glucan at the peak for this rabbit liver glycogen is considerably lower than in the model and this is balanced by more chains of d.p. > 20. Another profile of debranched chains of rabbit liver glycogen (Fig. 3; Akai et al., 1971) was similar. A third profile (for rabbit muscle glycogen) (Craig et al., 1988) (Fig. 3) gave a curve of the same shape, also with a lower peak value than the random model: however, although the maximum was at d.p. 14, the range of chain lengths was higher (up to > 100). In convolved curves of the model, the peak value was decreased little by increasing w to more than 6. With w set at the same value for all chain types, and ranging from 3 to 8 the maximum peak of numbers of chains was high (104) at $w = 3$. Then, as w increased the peak height initially decreased rapidly (79 at $w = 4$), but once w reached 6 only small further decreases occurred—to 60 at $w = 6$, 57 at $w = 7$ and 54 at $w = 8$. Those parts of the curves above d.p. 10 were very similar when w was 6 or more. When ECL_B was reduced to 6 and CL_A increased to 10 (maintaining ECL at 8) the calculated curve of relative weight of glucan versus d.p. retained the same general shape but the peak height was higher (82) at a d.p. of 12: when ECL_B was 7 the peak height was 68. If ECL_B was increased to 9 and ECL maintained at 8, with $w =$ 6 two peaks appeared (34 at d.p. 9 and 52 at d.p. 16) and this change was more pronounced when ECL_B was 10 (29 at d.p. 12 and 55 at d.p. 17). If w was increased to 7 when ECL_B was 9, there was a peak of 49 at d.p. 17 plus a point of inflection at d.p. 10 (height 34). Further increase of w to 8 gave a peak of 47 at d.p. 17 with an inflection at d.p. 10.

On the other hand, comparing a profile (Fig. 4) for debranched bovine liver glycogen (O'Shea & Morell, 1996) with the random dendrimeric model (Fig. 7) whereas the d.p. at which their peaks occur are similar, the experimental maximum is higher and there are no chains of d.p. > 40. Thus, although the distribution of chain lengths in the random dendrimeric model shows more similarities to the experimental debranching profiles than the regularly branched model, it still has significant differences. There were also differences between rabbit and bovine liver glycogens.

One possible mechanism giving these differences is that after formation of a new B_1 chain from an existing A chain the extension-branching system has a modified probability of continuing to extend and branch the newly-formed B chain rather than a different chain. If the overall proportions of A and B chains are a and b, in a polymer in which the probability of further extension and branching of the B chain newly-formed from an A chain is altered from that for extension and branching of other chains (the *adjusted* random dendrimeric model), the fractional distribution of chains becomes:

$$a, b(b_\delta, b_\delta a_\delta, b_\delta a_\delta^2, \ldots, b_\delta a_\delta^{k-1})$$

with $a_\delta = 1 - b_\delta$. The sequence of terms within the brackets converges: the sum of this series approaches a limit of one as k increases. From the apparent number of B_1 chains in an experimental curve and in the model an estimate of b_δ can be made since

$$\frac{bb_\delta}{b^2} = \frac{\text{apparent fraction of } B_1 \text{ chains in experimental curve}}{\text{fraction of } B_1 \text{ chains in model}}.$$

If $a_\delta > a$ the *adjusted* random dendrimeric model has fewer

Table 5
Numbers of chains at DP values for a random dendrimeric polymer with $a = 0.56$, ECL = 12, ICL = 6 and $T = 1260$

Type of chain	A	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_9	B_{10}	B_{11}	B_{12}
DP	12	19	26	33	40	47	54	61	68	75	82	89	96
No. of chains	706	244	137	76	43	24	13	8	4	2	1	1	0

B_1 chains and more chains of d.p. higher than B_2 compared to the random dendrimeric model. When an *adjusted* random dendrimeric model was derived, the curve of weight of glucan versus d.p. in Fig. 7 was obtained. It more closely resembles the experimental curve for rabbit liver glycogen than the random dendrimeric model, apart from a higher maximum d.p. Conversely if $a_\delta < a$ there are more B_1 chains and fewer B chains of higher d.p.in the *adjusted* random dendrimeric model than in the random model (cf. bovine liver glycogen, Fig. 4). Another reason for differences between the models and the experimental sructure is the possible operation of debranching enzyme (see Section 3.6).

The distribution of chains released by stepwise debranching by isoamylase acting in an *exo* manner is consistent with glycogen being approximately described by the *adjusted* random or random dendrimeric model. With oyster glycogen (Harada, Misaki, Akai, Yokobayashi, & Sugimoto, 1972; Palmer et al., 1983b) as the extent of debranching increased the average d.p. of released α(1-4) chains and the proportion of chains of higher d.p. increased. With random (or nearly random) branching the earlier a B chain is formed the lower its tier number and the more likely it is to carry a higher number of branches and hence be longer on debranching. The probability of a particular chain branching is inversely proportional to the number of chains already formed: chains formed later are more likely to be in a higher tier and have fewer branches.

The random dendrimeric model has a closer resemblance to the chain distribution of glycogen than the regularly branched model and its variations but the *adjusted* random dendrimeric model even more closely depicts the chain distribution in the naturally-occurring polymer.

3.6. Comparison of the random dendrimeric and transformed random dendrimeric models with waxy starches (rice, barley and maize)

Chain lengths for waxy starches have generally been found in the range 18–20 for CL, 11–13 for ECL, 5–6 for ICL and 14–15 for CCL. For a polymer with ECL = 12 ($ECL_B = CL_A$), ICL = 6, $a = 0.56$ and $T = 1260$ the calculated numbers of chains for the DP values of chain types are given in Table 5. Curves of the distribution of weight of glucan versus d.p. convolved from Gaussian distributions of the DP values and numbers of chains are shown in Fig. 8. With $w = 6$ the profile for the model has inflections at d.p. values corresponding to the DP of the types of chains. Experimental profiles have shown inflections and periodicity has been detected in the chain distributions of a number of amylopectins (Hanashiro et al., 1996). Comparisons of normalised elution profiles of weight of glucan of debranched chains of waxy barley and rice by a

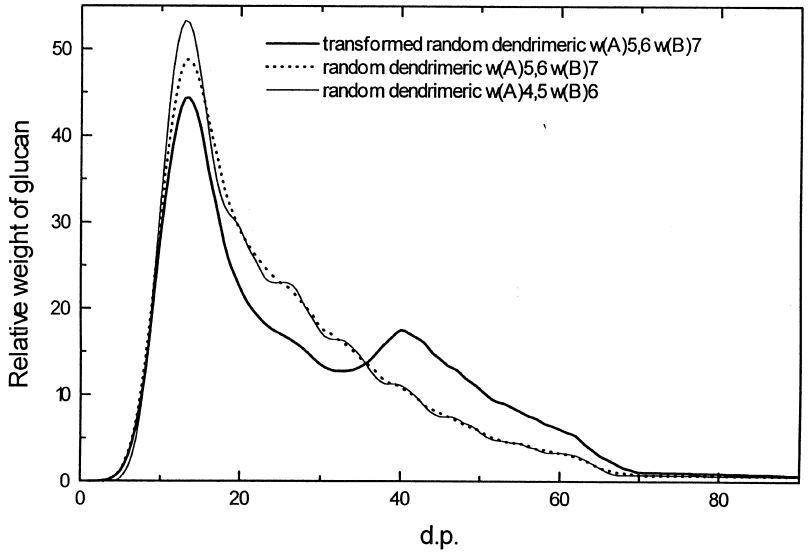


Fig. 8. Weight of glucans versus d.p. of α(1-4) chains in the random dendrimeric and *transformed* models of amylopectin ($a = 0.56$, $T = 1260$, CL = 19, ICL = 6, $ECL_B = CL_A = ECL = 12$: for *w*(A)4, 5*w*(B)6, 200 A chains convolved of DP and $w = 4$ plus 505 of DP 13 and $w = 5$; for *w*(A)5,6*w*(B)7, 200 A chains convolved at $w = 5$ plus 505 at $w = 6$).

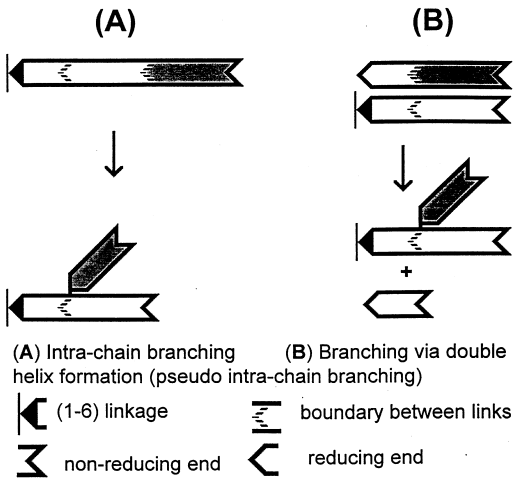


Fig. 9. Branching patterns of α(1-4) chains.

variety of chromatographic and detection techniques—with each other in Fig. 6 and with the random dendrimeric model in Fig. 8—show both similarities and differences. Debranched waxy maize has also been examined; by SEC on porous silica in neutral ionic solution with detection by refractive index (Yuan, Thompson, & Boyer, 1993) and on Fractogel in alkali with glucan estimation by phenol–sulphuric (Yun & Matheson, 1992). In the experimental curves the peak values were found in the d.p. range of 11–15, corresponding to the maximum due to A chains in the model. In the model the height of this peak was similar to that of some experimental samples (waxy rice) but higher than that of waxy barley. If ECL_B was reduced to 11 and CL_A increased to 13 (with $w = 7$) a calculated curve of relative weight of glucan versus d.p. with a similar general shape to the model that has both external chains equal to 12 was produced, but with a higher peak (57 at d.p. 14–15): if ECL_B was 10 an even higher peak resulted (66 at d.p. 15–16). Increasing ECL_B to 13 (with $w = 7$) and maintaining ECL at 12 gave two peaks of 43 at d.p. 12 and 28 at d.p. 21. When ECL_B was 14 these values became 39 at d.p. 11 and 29 at d.p. 23.

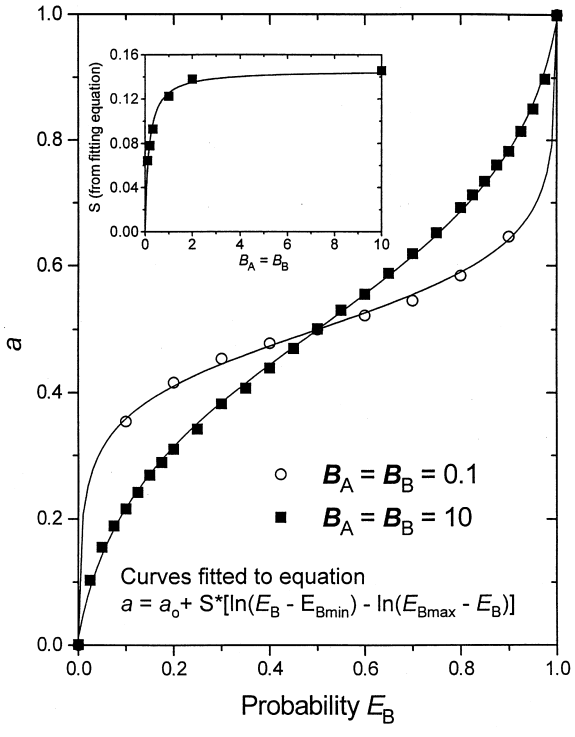


Fig. 10. The relationship between the probability of extension of B chains (E_B where $E_B = 1 - E_A$) and the fraction of A chains (a) at different probabilities of branching (B_A and B_B).

In the experimental profiles, an elevated level of amount of glucan occurs at a second smaller peak near a d.p. of 40 (B_4 chains in the model). This peak is at a higher d.p. than in the curves of models when ECL_B is increased to 13 or 14. In some, another small inflexion point was detected at an even higher d.p. (near 80) which, in SEC, because of the proximity to the void volume, may be due to a compression effect on chains of very high d.p., producing an artificial peak. The range of d.p. in the model extended to about 90. Two samples examined by SEC (waxy rice and waxy barley) gave small amounts of material near d.p. 100. The maximum d.p. for waxy rice chains separated in alkali by IEC-PAD was 71.

The sequence of fractions of chain types in the random dendrimeric model of amylopectin can be altered to give the *transformed* random dendrimeric model, which has a normalised curve of weight of glucan versus d.p. more closely approximating the experimental profiles containing this second smaller peak, by multiplying the values for B_1, B_2 and B_3 by a factor r, where $1 > r > 0$ and from B_4 to B_{12} by s, where $s \cong (1 - r + ra^3)/a^3$ to give the sequence

$$a, rb^2, rb^2a, rb^2a^2, sb^2a^3, \ldots, sb^2a^{11}.$$

In general when the second, smaller peak appears at B_{j+1} the sequence is

$$a, b(rb, rba, \ldots, rba^{j-1}, sba^j, \ldots, sba^{k-1})$$

where $s = (1 - r + ra^j)/a^j$.

Fig. 8 shows the normalised curve of weight of glucan versus d.p. of α(1-4) chains for this *transformed* random dendrimeric model where $r = 0.8$. The biosynthesis of amylopectin differs from that of glycogen in that it is subject to a diurnal-nocturnal light cycle. When nucleoside diphospho glucose substrate is in low concentration near dawn and dusk it may react more readily with longer B chains (with three or more branches) that are more accessible sterically, to produce a cluster model (Manners & Matheson, 1981; Robin et al., 1974) in which longer, more highly branched B chains link clusters. The role of debranching enzyme (James, Robertson, & Myers, 1995; Mouille et al., 1996) is another factor likely to produce differences from a random dendrimeric model. Processes such as selective debranching of interior chains on more highly branched B

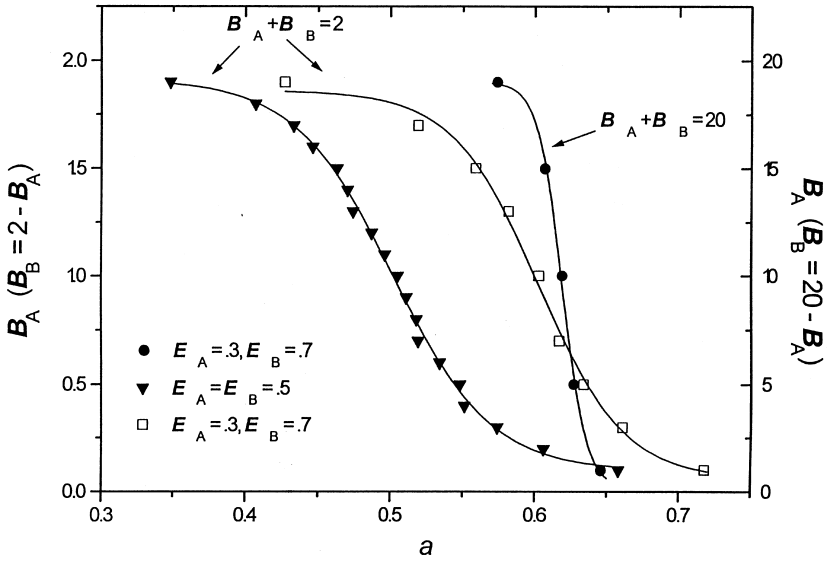


Fig. 11. Effect of $B_A + B_B$ on a in the random dendrimeric model.

chains or selective reduction in branching activity relative to extension could lead to another type of cluster model (French, 1984; Gallant, Bouchet, & Baldwin, 1997) in which long unbranched chains link the clusters. It has been proposed that the biosynthesis of amylopectin proceeds via modification of phytoglycogen by debranching (Erlander, 1998b).

The random dendrimeric model provides a better model for waxy amylopectin than any of the regularly branched models, but as it does not show the peak occurring at the d.p. of B_4 chains, the *transformed* random dendrimeric model, which does, is even closer.

3.7. Relationships between extension and branching probabilities and a, the tier structure and the various chain lengths in the random dendrimeric model

The first relationship studied was that between the relative probabilities of extension of an A or B chain (E_A and E_B) and the resulting a value, and then the effect of the branching probabilities (B_A and B_B) on this relationship. The program was run with $E_A + E_B = 1$, $T = 1000$ and 5 replications. Setting the B_A and B_B values equal and high (10–100) with either unlimited extension possible (program 1) or extension prohibited beyond three segments (program

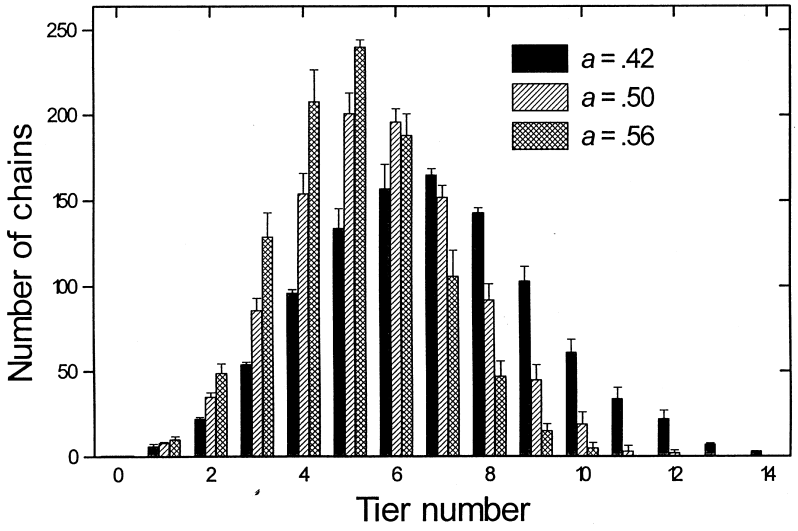


Fig. 12. Distribution of numbers of chains in tiers at a values of 0.42, 0.50 and 0.56 in the random dendrimeric model.

Table 6
Relationship between a and the mean, mode, median and maximum tier number (N) for the random dendrimeric model ($T = 1000$)

a	Mean number of tiers (m)	Mode	Median	Total number of tiers (N)	m/N
0.38	6.9	7	6.3	14	0.45
0.45	5.9	6	5.6	12	0.47
0.53	5.6	6	5.0	11	0.45
0.59	4.6	4	4.0	9	0.44
0.69	4.0	4	3.5	8	0.44
0.78	3.5	3	3.0	7	0.43

2) gave the relationship between E_B and a for both shown in Fig. 10. It can be fitted to the inverse of a Boltzmann equation:

$$a = a_0 + S \times [\ln(E_B - E_{B\min}) - \ln(E_{\max} - E_B)].$$

If the probability of branching was reduced the curve still fitted this equation but with decreased values for S. The shape of the curve changed so that for a values < 0.5 a lesser E_B value maintained the same a value and for a values > 0.5 a greater E_B was required. Both programs changed in the same way. The inset in Fig. 10 shows the change in S as B_A and B_B were modified (with $B_A = B_B$). The curve is the rectangular hyperbola of best fit to the points.

For program 1 (unlimited extension) if E_A and E_B were held constant and B_A and B_B varied within the same total of $B_A + B_B$, which was kept low, the a value changed within a limited range. Results for $E_A = E_B = 0.5$ and $E_A = 0.3$, $E_B = 0.7$ when B_A and B_B were varied within a total of 2 are illustrated (Fig. 11). When B_A and B_B were increased (to $B_A + B_B = 20$) the variation lessened.

The computed distribution of numbers of chains in each tier of the dendrimer generated by program 1 (unlimited extension) for a polymer of 1000 chains with a values of 0.42, 0.50 and 0.56 are plotted as histograms in Fig. 12. The single C chain at the reducing end of the molecule is considered to be tier 0. Table 6 gives the mean number of tiers (m), the median, the mode and the total number of tiers (N) and m/N for a range of values of a from 0.38 to 0.78. N, m the median and the mode all decrease as a increases. m/N is constant (0.45, s.d. 0.01) within this range of a values. Regardless of what extension and branching probabilities are applied, or whether the structure is generated by program 1 or program 2, the resulting a value determines m, the mode, the median and N. The actual values are dependent on T. Plots of m and the medians when $T = 1000$ for different a values are shown in Fig. 13. At a particular a value (from 0.38 to 0.99) the median was always lower than m. As a decreased N increased, from 3 at $a = 0.9$, to 8–9 at $a = 0.65$, to 11–12 when a was 0.5 and 13–14 when a was 0.4. The mode also increased as a decreased—from 3 at 0.9, to 4 at 0.65, 5–6 at 0.5 and 6–7 at 0.4.

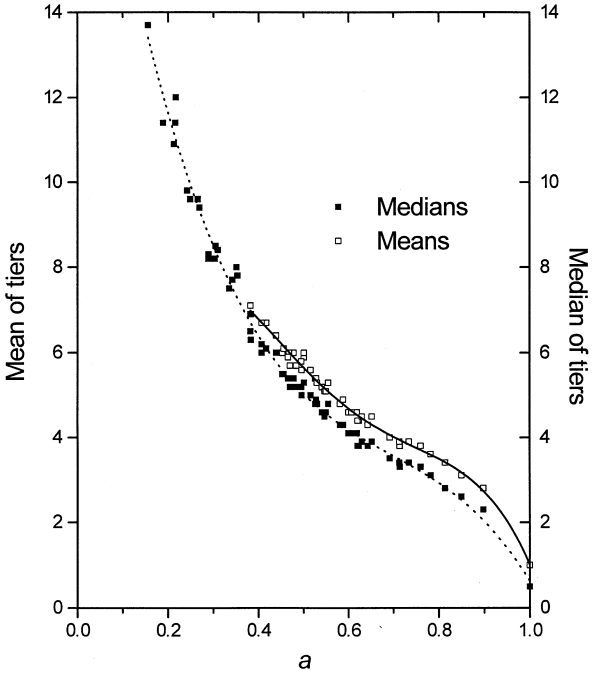


Fig. 13. Effect of a on mean tier number and the median tier number in the random dendrimeric model.

On random addition of chains to a dendrimer, as T increases the various parameters describing the tier structure initially increase rapidly and then more slowly, as shown for $a = 0.5$ up to 1400 chains in Table 7. Although the distribution of number of chains in tiers shows a general resemblance to a binomial distribution they differ significantly (χ^2 test) from the binomial $(0.55 + 0.45)^N$ having a skewness towards higher tier numbers. Branching of an A chain by intra-chain transfer produces a chain in the next higher tier (Fig. 14) whereas on branching of a B chain a new tier is not produced. The divergence from a binomial distribution for the tier structure would result from the few chains in the lower tiers having more opportunities to branch, whereas the greater number of chains formed later in middle tiers would have fewer opportunities. These tier patterns are quite different to the regularly substituted model, where

Table 7
Effect of T on parameters of the tier structure in the random dendrimeric model ($a = 0.5$)

T	63	250	500	750	1000	1250	1400
Maximum N	7	10	11	11	12	12	13
Mean	3.3	4.5	5.1	5.3	5.8	5.9	6.0
Mode	3	4	5	5	6	6	6
Median	2.9	3.9	4.3	4.5	5.3	5.4	5.5

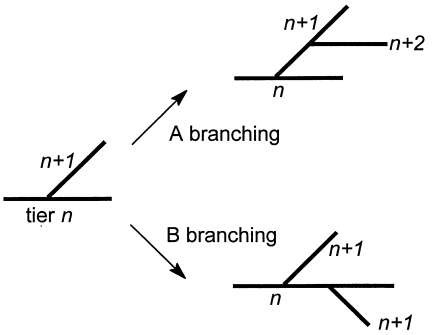


Fig. 14. Tier production on branching of A or B chains.

the number of chains in a tier continues to increase as the tier number increases. The tier structure in α(1-4) (1-6) glucans has not, at present, been established experimentally.

The average lengths of A chains (CL_A) and of segments of B chains {L_B, where $L_B = CL_B/(F + 1) = ((ICL + 1)F + ECL_B)/(F + 1)$} at different values of E_A and E_B, as well as B_A and B_B, expressed in relative terms as numbers of malto-oligosaccharyl building units for a model in which these are the same size, were calculated for program 1 (unlimited extension possible).

The results are shown in Fig. 15 for L_B and in Fig. 16 for CL_A versus a. Fig. 17 shows CL_B (where $CL_B = L_B(F + 1) = L_B(1 + b)/b$) and Fig. 16 CL (where $CL = aCL_A + bCL_B$.) versus a. The major factor affecting CL_A and CL is the probability of branching. When $B_A = B_B = 10$, L_B was constant for a from 0.01 to 0.9. At $B_A = B_B = 100$ it was also constant and very slightly lower (approximately 0.05 units): L_B increased somewhat when $B_A = B_B = 1$ and there was a large increase when $B_A = B_B = 0.1$. CL_A was relatively constant at $B_A = B_B = 10$ or 100 from $a = 0.01$ to 0.7, then decreased slightly. When $B_A = B_B = 1$ there was a

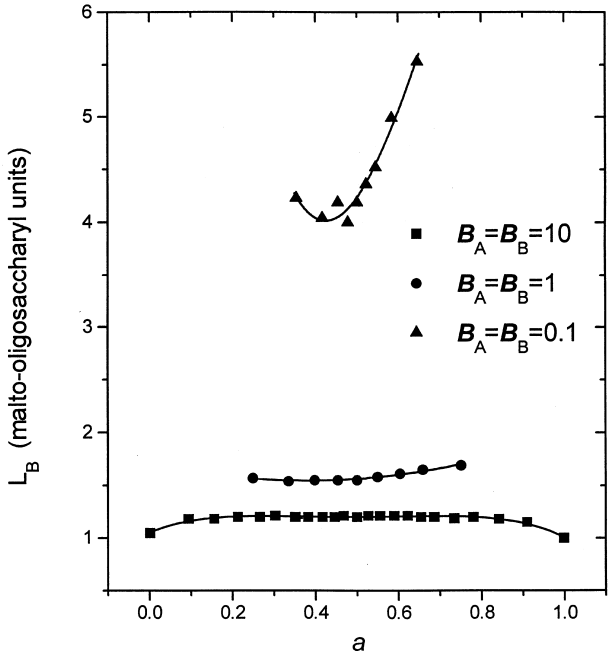


Fig. 15. Relationship between the average length of B chain segments (L_B) and a at different branching probabilities (B_A and B_B) in the random dendrimeric model.

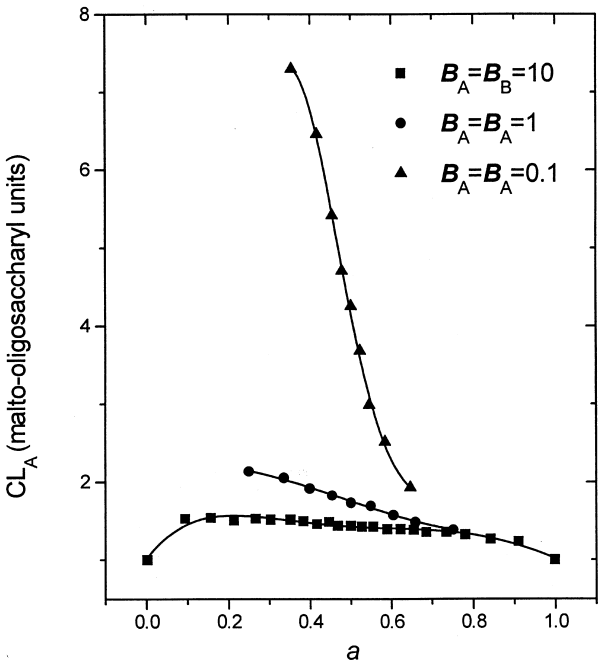


Fig. 16. Relationship between average length of A chains (CL_A) and a at different branching probabilities (B_A and B_B) in the random dendrimeric model.

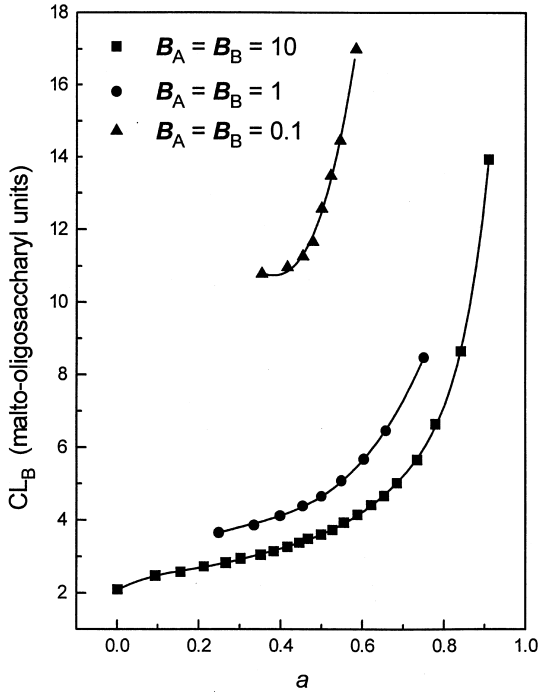


Fig. 17. Relationship between average lengths of B chains (CL_B) and a at different branching probabilities (B_A and B_B) in the random dendrimeric model.

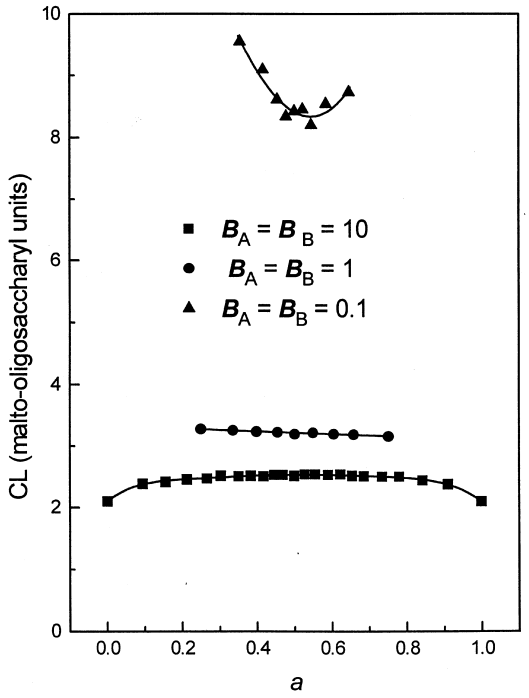


Fig. 18. The relationship between average overall chain length (CL) and a at different branching probabilities (B_A and B_B) in the random dendrimeric model.

small decrease between $a = 0.44$ and 0.78, but at $B_A = B_B = 0.1$ there was a large decrease from $a = 0.45$ to 0.52.

CL_B increased as a increased and was higher the lower the probability of branching. At a particular probability of branching (Fig. 18) CL was similar and increased as B_A and B_B decreased. Experimentally CL increased through the sequence rabbit liver glycogen, phytoglycogen, waxy, normal and amylose-extender genotypes of maize (Matheson, 1996) and this was associated with increases in all chain length averages (ECL, ICL and CCL).

3.8. Normal and amylose extender amylopectins

Comparisons of calculated α(1-4) chain distributions of models with experimental profiles of normal and amylose-extender amylopectins are more difficult because of problems of ensuring that on fractionation of amylopectin and amylose all of the latter has been removed and that the recovery of amylopectin is complete, as well as the possible presence of structures intermediate between amylopectin and amylose (MacGregor & Morgan, 1984; Matheson & Welsh, 1988; Matheson, 1990; Ong et al., 1994). Also, the range of lengths of α(1-4) chains can be much increased (due to higher ECL and ICL) (Matheson, 1996) so that on SEC it is difficult to find a matrix that efficiently fractionates the entire range of molecules and does not give a false peak at the void volume due to compression of chains with higher d.p. values. The small amounts of fractions of high d.p. causes uncertainty in quantitation by methods that estimate reducing-end function, such as PAD and fluorescent labelling. Also, as chains increase in d.p. there is an increased chance of their precipitation, unless an alkaline solvent is used.

The random dendrimeric model is consistent with one aspect of α(1-4)(1-6) glucans, the increase in CL, ICL, ECL and CCL from waxy through normal to amylose-extender maize genotypes (Matheson, 1996). These vary in starch branching capacity due to the presence of different enzymic forms (Bhattacharyya, Smith, Ellis, Hedley, & Martin, 1990; Safford et al., 1998). In the random dendrimeric model, as the probability of branching is reduced from 10 to 0.1 (with $B_A = B_B$) CL_A, CL_B and CL and hence CCL (Section 3.7, Figs. 16–18) all increase. If B_A and B_B are increased from 10 to 100 only a slight decrease in these various chain lengths occurs. A decreased probability of branching could also be associated with the higher levels of amylose in genotypes with longer chain lengths of amylopectin, when there would be an increased chance that unbranched or lightly branched, short-chain precursor molecules, instead of branching, would be lengthened sufficiently to precipitate.

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