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Heterogeneity in Branching of Amylopectin

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SUMMARY

The angular dependence of scattered light from amylopectin and its β-limit dextrin, the mean square radius of gyration and the molecular weights M_w and M_n have been calculated on the basis of the cascade branching theory for the homogeneously branched model by Meyer & Bernfeld (1940) (Model I) and for the two heterogeneously branched structures suggested by French (1972) (Model II) and by Robin et al. (1974, 1975) (Model III). The calculations take into account the particularities of topology in branched molecules and the experimentally determined ratio of the number of A- and B-chains, A/B = 1. Furthermore, an average branching density of 4% and an interconnecting chain length of $\bar{n}_{i2} = 22$, found by gel permeation chromatography (GPC) after debranching, were used. The constraints lead to the conclusion that amylopectin is heterogeneously branched. Densely branched clusters containing 3·22 branching units are interconnected by longer chains of 22 units in length. Comparison of the calculated angular dependence of light scattering with measurements from a maize amylopectin β-limit dextrin in 1 N NaOH solution gives strong evidence for a modified Robin-Mercier model. The modification consists of the conclusion that the interconnecting chains are preferentially B-chains, such that these chains carry on the average 1·4 clusters, while Robin and Mercier assume exactly 2 clusters. Our result is in agreement with the distribution of chain length found after debranching the amylopectin β-limit dextrin.

INTRODUCTION

For quite some time polymer scientists have been considering models for branched structures (Stockmayer, 1943, 1944). Amylopectin (AP)

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was among the first molecules they considered, and, eventually, a number of models describing the AP structure have been suggested. Today only three of them are discussed seriously: the Meyer–Bernfeld model (I) (Meyer & Bernfeld, 1940) which describes AP as a homogeneously branched molecule, the heterogeneously branched French model (II) (Kainuma & French, 1971, 1972; French, 1972) and the Robin–Mercier model (III) (Gunja-Smith *et al.*, 1970; Robin *et al.*, 1974, 1975). Models II and III are similar and differ in detail only. The homogeneous Meyer–Bernfeld model is the simplest *statistical* model which is compatible with the average branching density of AP. It was considered unanimously as a valid representation of the AP structure, until 1970 when the two debranching enzymes pullulanase and isoamylase became known and were applied to amylopectin, giving clear evidence that this storage polysaccharide must be heterogeneously branched, i.e. there exist regions in the molecules where several branching points are clustered together and others where branching points are less frequent than the average branching density would suggest.

All model suggestions so far were made on the basis of enzymic degradation experiments combined with the analysis of the oligomeric products obtained. A few cases only have been considered where the macromolecule is undamaged (Erlander & French, 1956; Zimm & Stockmayer, 1949; Burchard, 1972; Burchard *et al.*, 1975). The various structural elements should exhibit a marked influence on the properties in solution, i.e. polydispersity, overall molecular dimensions, hydrodynamic behaviour and internal mobility. For this reason the authors became interested in the structure and properties of the native, non-degraded AP, which can be determined by light scattering (LS). These LS measurements, which were carried out at the wavelengths of 647 nm (red), 546 nm (green), 436 nm (blue) and 365 (near u.v.) and at five concentrations, allowed the determination of the molecular weight M_w, the mean square radius of gyration $\langle S^2 \rangle_z$ and a recording of the angular dependence of the scattered light, i.e. the particle scattering factor $P_z(q)$, over a wide range of the scattering vector $q = (4\pi/\lambda) \sin\theta/2$, where θ is the scattering angle. The particle scattering factor is closely related to the local segment density distributions in the molecule which are different in the three models mentioned above.

We carried out statistical model calculations for the angular dependence of the scattered light, where the specific details of the various proposed models were taken into consideration (Burchard & Thurn,

1985). The purpose of these calculations was to examine whether the variations in the models produce a significant change in the particle scattering factor, which may allow confirmation of one of these models and the exclusion of the two others. Complete separation of the highly branched AP from the essentially linear amylose chains in starch is difficult (Banks & Greenwood, 1975). Since the presence of linear chains has a big influence on the angular dependence of the scattered light and falsifies the conclusions drawn from LS measurements, we, therefore, first prepared the β-limit dextrin (β-LD) by applying β-amylase to AP, and subsequently carried out the LS measurements. By action of the β-amylase all linear chains became degraded to maltose and the outer chains in AP were reduced to stubs of two to three anhydroglucose units in length. The maltose so produced could be easily removed by dialysis.

TOPOLOGY OF BRANCHED CHAINS

Most of the models suggested for AP have been formulated more or less intuitively, and the possibility that the various structural parameters might be correlated (for instance the branching density and the length of inner and outer chains) has not been taken into consideration.

Compared to linear chains, where the problems of structure appear 'trivial', the structural problems in branched molecules are rather complicated, and it becomes essential here to think about the topology of branching. The conclusions arising from topology are then incorporated in the following different models.

Figure 1 shows three different models which have been proposed previously for the AP branching structure. In these cases, the decision in favour of the Meyer–Bernfeld model was made on the ratio of A/B-chains, where an A-chain is an outer chain linked by only one α-(1,6)-glycosidic bond to a B-chain. This assumes that an A-chain carries no branching units. A B-chain is always branched and may carry shorter B-chains and is linked by an α-(1,6)-glycosidic bond to a longer B-chain. The ratio of A- to B-chains is characteristically different in various structures. By counting the chains, we see in the Meyer model (Meyer & Bernfeld, 1940) a ratio of A/B = 1. The comb-like model suggested by Staudinger & Husemann (1937) has one B-chain only, and the others are all A-chains. Thus A/B tends to infinity with increasing molecular

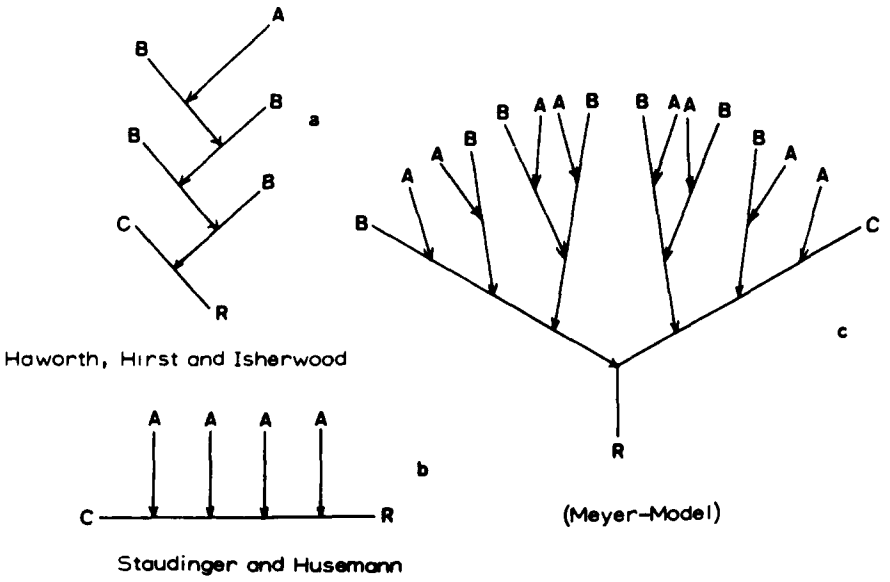

Fig. 1. Branching structure for amylopectin according to: (a) Haworth *et al.* (1977) (laminated structure); (b) Staudinger & Husemann (1937) (comblike structure); and (c) Meyer & Bernfeld (1940) (tree-type structure). Only model (c) gives the correct ratio A:B = 1. C is the only B-chain in the molecule with the reducing endgroup R. An A-chain is always an unbranched outer chain. B-chains are always branched, but parts of them also form outer chains.

weight. In the 'laminated' structure (Bathgate & Manners, 1966; Manners & Matheson, 1981) the opposite behaviour is obtained, i.e. $A/B \rightarrow 0$.

The pictures appear very simple, but the difficulty is in distinguishing by analytical chemical means an A- and a B-chain. The determination of the A/B-ratio was made conclusively possible with the discovery of the debranching enzymes pullulanase and isoamylase, and their application to AP by Manners and co-workers (Bathgate & Manners, 1966; Manners & Matheson, 1981).

In the following, we review briefly the procedures for determining the two essential constraints, i.e. (I) the ratio A/B of the number of A- to B-chains and (II) the average branching density. These techniques are widely known but are repeated here to avoid extensive cross-referencing.

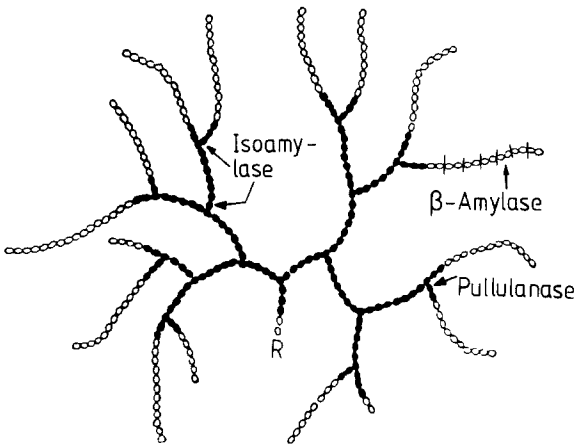

Fig. 2. Schematic structure of amylopectin and action of the enzymes β-amylase, isoamylase and pullulanase.

The debranching enzymes act in the manner depicted in Fig. 2. Isoamylase breaks α-(1,6)-glycosidic bonds only if the chain carries at least three glucose units before a branching point, whereas pullulanase is able to debranch shorter α-(1,4)-chains. For the same reason as outlined in the introduction, Manners did not use AP but used its β-limit dextrin. The β-amylase stops cleaving maltose from the non-reducing chain ends at two or three glucose units before a branching point, depending on whether the outer chain had an even or odd number of repeating units. Therefore β-LD consists of 50% outer chains with two and 50% of those with three glucose units (the assumption of an equal number of even and odd outer chains is a generally accepted hypothesis). Consequently, isoamylase (I) breaks all α-(1,6)-bonds connecting B-chains and half of all A-chains. Determination of the end groups from the isoamylase treatment results in (A/2 + B)-chains. Using both enzymes in one experiment (II), all A- and all B-chains become detached and end group determination now yields the number of (A + B)-chains. Therefore the ratio A/B is:

$$A/B = 2(II - I)/(2I - II) \quad (1)$$

In all experiments which Manners & Matheson (1981) carried out with AP from various sources, they found a ratio of A/B-chains of

$$A/B = 1 \pm 0{\cdot}1 \quad (2)$$

They also observed that contradictory results were sometimes found, possibly arising from the fact that a large excess of enzyme had to be used.

The branching density is reliably determined by methylating all free hydroxyl groups, followed by an acid hydrolytic total degradation of the permethylated β-LD. The monomeric units have only OH-groups in those positions which were blocked in the polymer by glycosidic bonds and consequently were not accessible for methylation. Characterisation of the monomers by gas chromatography gives the following different fractions:

Fraction I: Glucose units methylated at positions C2, C3 and C6, coming from the α-(1,4)-linked chains.

Fraction II: Glucose units methylated at positions C2 and C3, coming from the branching points (α-(1,4)- and α-(1,6)-linked).

Fraction III: Glucose units methylated at positions C2, C3, C4 and C6, coming from the end groups.

The intensity of the various peaks gives the degree of branching according to the following equation

$$DB = \mathrm{II}/(\mathrm{I} + \mathrm{II} + \mathrm{III}) \tag{3}$$

THE RELATIONSHIP BETWEEN BRANCHING DENSITY AND LENGTH OF BRANCHES

Homogeneous branching

If we let K be the number of branching points per macromolecule, and $\bar{n}_0$ and $\bar{n}_i$ be the average chain length of the outer and inner chains (see Fig. 3), then the branching density DB is defined as the number of branching points K per molecule (i.e. per P_n). The number average degree of polymerisation P_n can be calculated from the number of branching points K and the average length of the inner and outer chains, and is given by

$$P_n = \bar{n}_0(K + 1) + \bar{n}_i(K - 1) + \bar{n}_r \tag{4a}$$

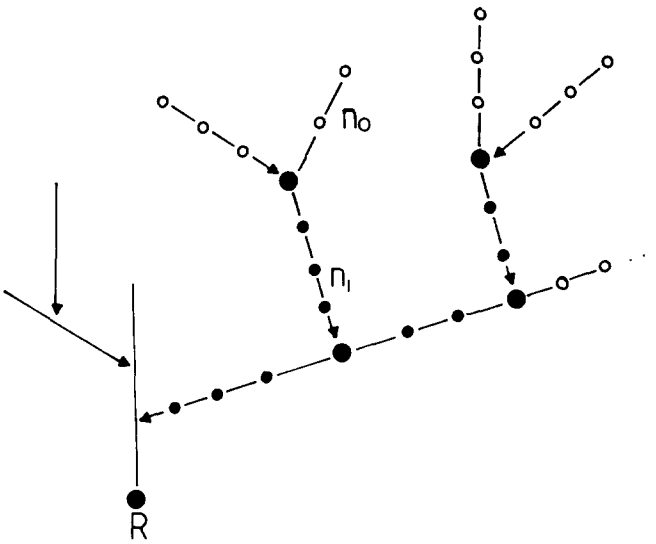

Fig. 3. Schematic structure of the modified Meyer model. The internal structure corresponds to the original Meyer model, which can be generated by ABC-polycondensation (Burchard, 1972). ● represents a three-functional branching unit. The outer chains (—○—○—) have a degree of polymerisation $\bar{n}_0 = 13{\cdot}5$ for the AP, or $\bar{n}_0 = 2{\cdot}5$ for the β-LD. The inner chain length (—●—●—) $\bar{n}_i = 11{\cdot}5$ is the average length between two branching points; → denotes an α-(1,6)-glycosidic bond.

which for $K \gg 1$ becomes

$$P_n \to K(\bar{n}_i + \bar{n}_Q) \tag{4b}$$

$\bar{n}_r$ being the only outer chain with a reducing end group. Using eqn (4) one can find the branching density DB

$$DB = K/P_n = 1/[(\bar{n}_i + \bar{n}_0) + (\bar{n}_0 - \bar{n}_i + \bar{n}_r)/K] \to 1/(\bar{n}_i + \bar{n}_0) \tag{5}$$

As already mentioned, the branching density can be determined by experiment, but with this parameter only the *sum* of an inner and outer chain $\bar{n}_i + \bar{n}_0$ can be calculated. In order to determine $\bar{n}_i$ and $\bar{n}_0$ separately one has to measure, on the one hand, the mass of β-LD and, on the other hand, the mass of AP used before β-amylase digestion. X is given by:

$$X = (\text{mass})_{\beta\text{-LD}}/(\text{mass})_{\text{AP}} = P_{n\beta\text{-LD}}/P_{n\text{AP}} \tag{6}$$

where the second equivalence is a generally accepted approximation (Banks & Greenwood, 1975). Making use of eqns (4) and (5), one finds

$$X = (\bar{n}_i + 2{\cdot}5)/(\bar{n}_i + \bar{n}_0) \tag{7a}$$

or

$$X = DB(\bar{n}_i + 2{\cdot}5) \tag{7b}$$

$$\bar{n}_i = (X/DB - 2{\cdot}5) \tag{8a}$$

$$\bar{n}_0 = (1/DB) - \bar{n}_i \tag{8b}$$

The density of branching varies for the different APs from $DB = 0{\cdot}036$ to 0·045 (Heyns, 1949), and a fraction $X = 0{\cdot}53$–$0{\cdot}56$ is usually found (Banks & Greenwood, 1975). In our calculations, we used values of $DB = 0{\cdot}04$ and $X = 0{\cdot}56$. For AP and its β-LD the values listed in Table 1 are obtained.

Heterogeneous branching

The relationship of eqns (4) to eqns (8) is general and holds also for heterogeneously branched APs. However, $\bar{n}_i$ is now the average over two types of inner chain lengths. Letting $\bar{n}_{ic}$ be the average inner chain length in a cluster and $\bar{n}_{i2}$ the average length of chains connecting two clusters – furthermore, the number of branching points Z_k in a cluster may be introduced and the average number of chains originating in one cluster leading to another cluster, i.e. the average number of chains $\bar{f}$ connecting two clusters – then (see Fig. 4):

$$\bar{n}_i = (\bar{f}\bar{n}_{i2} + (Z_k - 1)\,\bar{n}_{ic})/(Z_k + \bar{f} - 1) \tag{9}$$

TABLE 1
Branching Density *DB* and the Resulting Inner and Outer Average Chain Length $\bar{n}_i$ and $\bar{n}_0$ for AP and its β-LD

	AP	*β-LD*
$DB^{-1} = \bar{n}_i + \bar{n}_0$	25	14
$\bar{n}_i$	11·5	11·5
$\bar{n}_0$	13·5	2·5

or

$$Z_k = (\bar{f}\bar{n}_{i2} - (\bar{f} - 1)\,\bar{n}_i - \bar{n}_{ic})/(\bar{n}_i - \bar{n}_{ic}) \tag{10a}$$

From the general theory of branching, gelation takes place, when

$$(\bar{f} - 1) = 1 \tag{11}$$

which is Flory's gel condition (Flory, 1941, 1953). Gelation does not occur in AP, though the weight average molecular weight is very high. Thus $\bar{f}$ is very close to 2 and eqn (10a) reduces to

$$Z_k = (2\bar{n}_{i2} - \bar{n}_i - \bar{n}_{ic})/(\bar{n}_i - \bar{n}_{ic}) \tag{10b}$$

From the three quantities on the right of eqn (10b), $\bar{n}_i = 11{\cdot}5$ was already obtained from eqn (9), $\bar{n}_{i2}$ was determined by Robin *et al.* (1974, 1975) and in our own debranching experiments (Thurn & Burchard. 1984; Eschwey *et al.*, 1985) to have values between $\bar{n}_i = 20$–25, while the chain length $\bar{n}_{ic}$ in a cluster is unkown to date (it may range from 2 to 4). Table 2 gives a list of the number of branching points in a cluster for $\bar{n}_{ic} = 2$, 3 and 4, and a length of $\bar{n}_{i2} = 22$. The number of chains in a cluster of the type in Fig. 4 is $Z_k + 1$. (Actually $Z_k + 1$ is the number of outer chains; the chains connecting two clusters are also outer chains, which became longer chains by coupling two clusters. In

TABLE 2

Number of Branching Points Z_k in a Cluster Calculated by eqn (10b) for a Chain Length $\bar{n}_{i2} = 22$ (connecting two clusters) and Various $\bar{n}_{ic}$ (inner chains in a cluster)[a]

$\bar{n}_{i2} = 22$	$\bar{n}_{ic} = 2$	$\bar{n}_{ic} = 3$	$\bar{n}_{ic} = 4$
Z_k	3·22	3·59	3·97
P_{nc}	7·46	11·88	16·88
P_{wc}	34·80	75·37	127·32

[a] The total number of chains per cluster is $Z_k + 1$. P_{nc} and P_{wc} denote the number and weight average degree of polymerisation of a cluster. The outer chains of such a cluster have been assumed to be of the same length as the internal chains, i.e. $\bar{n}_{oc} = \bar{n}_{ic}$.

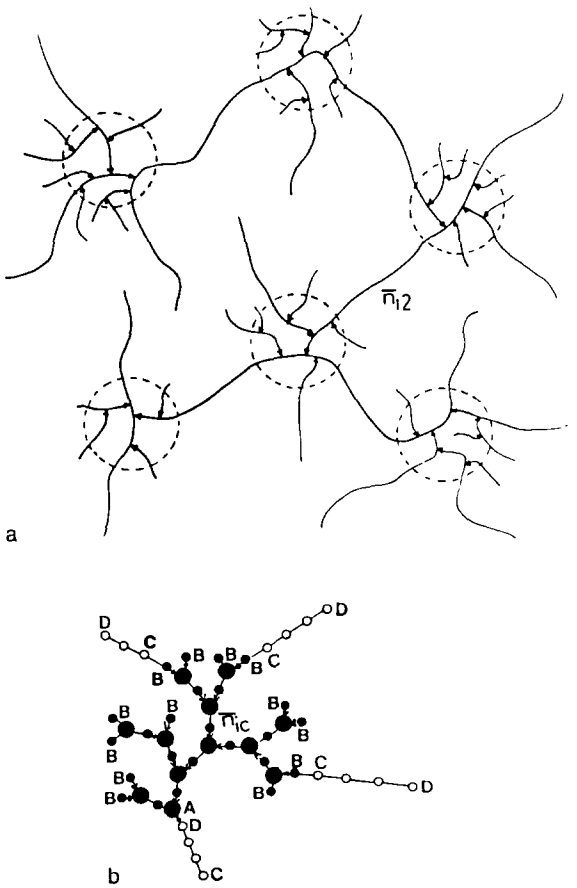

Fig. 4. (a) Model of a heterogeneously branched macromolecule and (b) detail of cluster-structure. C—D, interconnecting chains of the length $\bar{n}_{i2}$; ●, the Z_k branching points in a cluster.

the β-LD only the free ended outer chains can be attacked by β-amylase.) This gives, for $\bar{n}_{i2} = 22$ and $\bar{n}_{ic} = 2$, on the average 4·22 chains per cluster which is close to the suggested value of Robin *et al.* (1974, 1975). The authors give in their original paper a value of 8 to 9 which, however, is the average number of chains for *two* clusters, since in their model an interconnecting chain carries two clusters.

In all further calculations we assumed the highest possible branching density of 50% for the clusters, i.e. $\bar{n}_{ic} = 2$. The cluster has been calcu-

lated according to the AB_2 model (see Fig. 4(b)) where A can react with B only. Then P_w is given as

$$P_w = 1 - \alpha^2[(1-p)^2 + p^2]/(1-\alpha)^2$$

where p is the branching probability and

$$\bar{n}_{ic} = 1/p$$

Evidently the highest branching probability is 0·5 and yields $\bar{n}_{ic} = 2$.

General remarks on the proposed models

The main difficulty is in incorporating a heterogeneity in branching under the constraint of A/B = 1. The homogeneous Meyer model automatically meets this condition. Thus, the natural extension of the Meyer model involves the choice of clusters which have the same internal structure but are smaller in size. The ratio of A/B = 1 is not altered, if these clusters are interconnected by longer B-chains.

All model calculations were carried out on the basis of the cascade branching theory (Burchard, 1983), using the idea of a polycondensation of ABC-type monomers (Fig. 5). The two molecular weight averages M_w and M_n, the mean square radius of gyration $\langle S^2 \rangle_z$ and the particle scattering factor $P_z(q)$ have been calculated for the three models mentioned in the introduction and are discussed in the literature (Burchard & Thurn, 1985). All model calculations are valid for AP as well as for its β-LD, since only the outer chain length $\bar{n}_0$ has to be changed.

Meyer model

There is no actual need for a statistical calculation of this homogeneously branched model, since the enzymic degradation experiments have given clear evidence for the heterogeneity of branching. Nevertheless, calculation of this model appears useful, since it is used as a basic model for the heterogeneously branched models.

Furthermore, we wished to develop a model which holds for both the AP and its β-LD, and where only the average length of the outer chains has to be changed. This is accomplished by calculating a Meyer model, which is followed by coupling a branching unit with certainty onto the end of the outer chains (modified Meyer model). This branch-

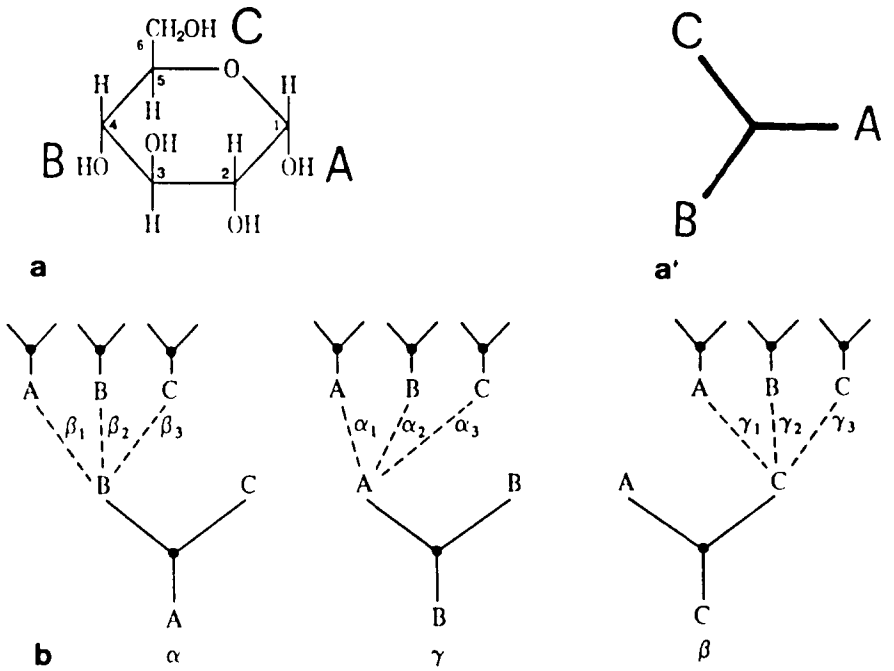

Fig. 5. (a) The monomeric unit of AP is glucose, which may for simplicity be contracted to the graph (a'). In AP group A can be linked only to group B or C; all other reactions are excluded. (b) The various reaction probabilities for the functional groups A, B and C of a monomeric unit in the *n*th generation, when group A or B or C is linked to a unit in the previous generation. The Greek letters denote the various link probabilities.

ing unit carries two other outer chains (see Fig. 3). The original Meyer model was calculated in 1972 by Burchard (1972). The principles involved in calculating M_w and M_n, radius of gyration, $\langle S^2 \rangle_z$, and particle scattering factor, $P_z(q)$, have been outlined elsewhere (Burchard & Thurn, 1985) (see Fig. 6).

A plot of the reciprocal particle scattering factor ($1/P_z$) as a function of $u^2 = q^2 \langle S^2 \rangle$ is shown by the pair of curves (a) in Fig. 7. The dashed line corresponds to AP and the full line to β-LD. The particle scattering factor represents the angular dependence of the scattered light which is normalized at zero angle to $P_z(q = 0) = 1$; $q = (4\pi/\lambda) \sin \theta/2$ is the value of the scattering vector, with λ the wavelength in the medium

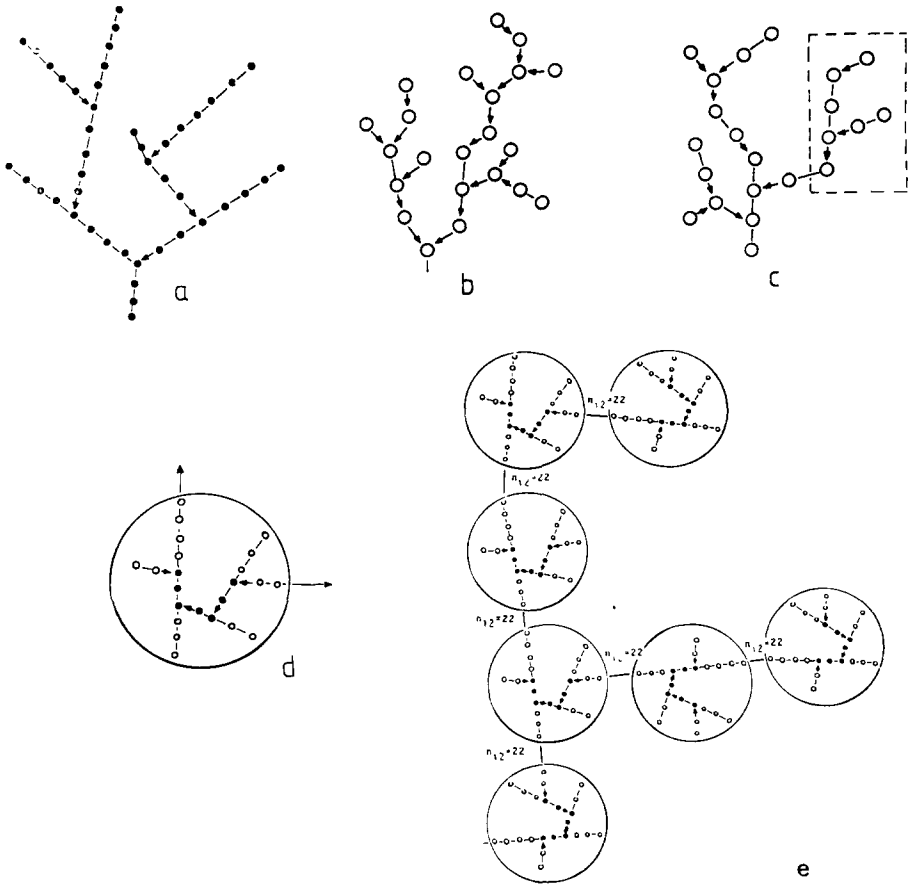

Fig. 6. (a) Meyer model (homogeneous); (b) French model (heterogeneous); (c) modified Robin-Mercier model (heterogeneous). The filled circles (●) in graph (a) (the term graph is here used in the sense of graph theory) denote individual glucose units where the arrow (→) indicates α-(1,6) branching points and the edges (——) α-(1,4)-glucose bonds. Graphs (b) and (c) are contracted graphs which are explained in more detail in graphs (d) and (e) (for further information see text).

and θ the scattering angle. $u^2 = \langle S^2 \rangle q^2$ is used as a variable on the abscissa, since all particle scattering factors, irrespective of the model, start with the initial slope of 1/3. The calculated curves show a strong upturn and disagree markedly with experimentally obtained curves (Erlander & French, 1956; Burchard *et al.*, 1975), as could be antici-

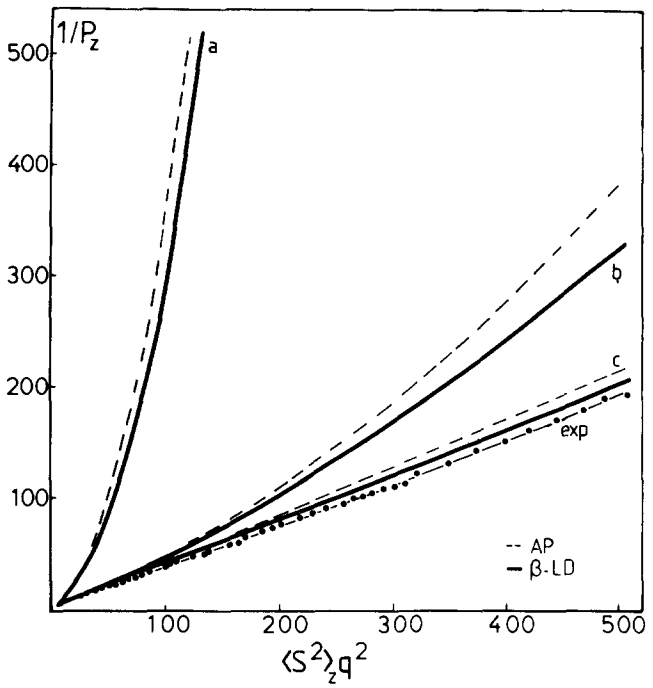

Fig. 7. Reciprocal particle scattering factor, $1/P_z$, as a function of $u^2 = \langle S^2 \rangle_z q^2$ for the three models given in Fig. 6 compared with $1/P_z$ measured by static LS. The dashed lines correspond to AP, the heavy lines to the β-LD; —●—●—, experimental curve (Burchard *et al.*, 1975; Eschwey *et al.*, 1985).

pated from the enzymic debranching results. In addition the molecular weight averages M_w and M_n, the mean square radius of gyration $\langle S^2 \rangle_z$, and the effective bond length b have been calculated. Table 3 gives the data for M_w/M_n, and b for a β-LD with $M_w = 333 \times 10^6$.

French model

As already outlined, in the French model (see Fig. 6) the clusters have the internal structure of the Meyer model and are linked via chains of length $\bar{n}_{i2} = 22$. In this figure the graphs (b) and (c) represent contracted graphs in which the open circles (o) no longer represent individual glucose units, but symbolize a densely branched cluster, the detailed structure of which is shown in graph (d). The lines represent

TABLE 3
Calculated and Experimental Values for M_w, M_n, $\langle S^2 \rangle_z$, M_w/M_n and Effective Bond Length, b, for the β-LD of the Indicated Models

	Experiment	*Calculated*		
		Meyer	*French*	*Robin-Mercier*
M_w	$3{\cdot}33 \times 10^8$	$3{\cdot}33 \times 10^8$	$3{\cdot}33 \times 10^8$	$3{\cdot}33 \times 10^8$
$\langle S^2 \rangle_z^{0{\cdot}5}$	260 nm	–	–	–
M_n	–	$8{\cdot}22 \times 10^5$	$9{\cdot}22 \times 10^4$	$3{\cdot}89 \times 10^4$
M_w/M_n	–	$4{\cdot}05 \times 10^2$	$3{\cdot}61 \times 10^3$	$8{\cdot}56 \times 10^3$
b	–	4·36 nm	2·41 nm	0·79 nm

linear α-(1,4) chains of $\bar{n}_{i2} = 22$ glucose units in length connecting the clusters of type (d) and are not individual bonds. The arrows indicate that these chains are linked by α-(1,6) bonds to the cluster (d). Graph (d) represents an expanded graph of the internal clusters of (b) and (c). Here the filled circles belong to the internal structure of the cluster, while the open circles belong to the outer chains ($\bar{n}_0 = 13{\cdot}5$ or 2·5 for AP and its β-LD, respectively). The arrows outside the big circle indicate the chains connecting two clusters with the length $\bar{n}_{i2} = 22$.

The difference between (b) and (c) is demonstrated in graph (e) by the detailed structure of a section of the molecule as indicated by the dotted line. A 'side' chain has in this case a length $\bar{n}_{is} = \bar{n} \,.\, \bar{n}_{i2} = \bar{n} \,.\, 22$, where $\bar{n}$ is the average number of clusters per side chain. NB: In the original Robin-Mercier model $\bar{n} = 2$ is *not an average value*; in the modified Robin-Mercier model $\bar{n}$ is an *average value* and was found to be $\bar{n} = 1{\cdot}4$.

For the branching density in the clusters we assumed $DB = 0{\cdot}5$ (i.e. $\bar{n}_{ic} = 2$), which is the highest possible branching density. This implies 4·22 chains per cluster on the average. These data are sufficient for a unique description of the cluster structure which is given in the two last entries of Table 2 (e.g. P_{wc}, P_{nc}). On each of the $Z_k - 1$ chains a linear chain of the length $\bar{n}_0$ (i.e. 11·5 for AP and 2·5 for β-LD) is coupled statistically. These modified clusters are then interconnected by linear chains of length $\bar{n}_{i2} = 22$. This is achieved by coupling both

ends of the linear chain onto the remaining free two end groups of the cluster (Burchard *et al.*, 1981).

The resulting data are given in Table 3 and $1/P_z$ is again plotted in Fig. 7 (pair of curves (b)). The agreement with the experiment is better, but not perfect.

Modified Robin-Mercier model

A perfect agreement was not expected in the previous case since our own debranching experiments gave linear α-(1,4)-glycosidic linked chains with a degree of polymerisation up to 75–90 (see Fig. 8). These findings indicate that the coupling of clusters does not occur at random between the longer outer chains, but it is preferentially a B-chain which accomplishes the coupling. We thus modified the French model, assuming linear B-chains which on the average carry $\bar{n}$ clusters, i.e. $\bar{n}_{is} = \bar{n} \,.\, \bar{n}_{i2} = \bar{n} \,.\, 22$. All other assumptions remain the same as used for the French model, with the exception that the probability of cluster inter-

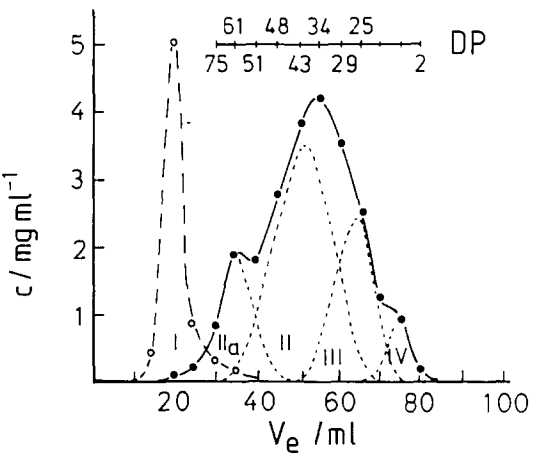

Fig. 8. GPC elution diagram of debranched β-LD. Four peaks referring to different linear chain length can be identified: Peak IV, stubs of outer chains $\bar{n}_0 = 2$–7 (not completely resolved); peak III, $\bar{n}_{i2} = 22$; peak II, $\bar{n}_{i2} = 2 \,.\, 22 = 44$; peak IIa, $\bar{n}_{i2} = 3 \,.\, 22 = 66$; peak I, elution diagram of the non-debranched β-LD. Debranching was performed with pullulanase and the oligomers were fractionated on Bio-rad P10 gel. DP denotes degree of polymerisation.

connection has to be reduced in order to avoid gelation. This model differs from the suggestion by Robin *et al.* (1974, 1975) in two points: (i) These authors assumed for the interconnecting B-chains a length, of *exactly* $2 . \bar{n}_{i2}$, while in our model the number of coupled chains $\bar{n}$ was changed systematically. (ii) This number $\bar{n}$ is in the present model an *average* value, i.e. there may be shorter and also longer chains effective in the crosslinking. The size distribution is simulated by a linear polycondensation of chains of the length $\bar{n}_{i2}$. The difference between the original Robin-Mercier model and our modification is schematically shown in Fig. 9 for two values of $\bar{n}$.

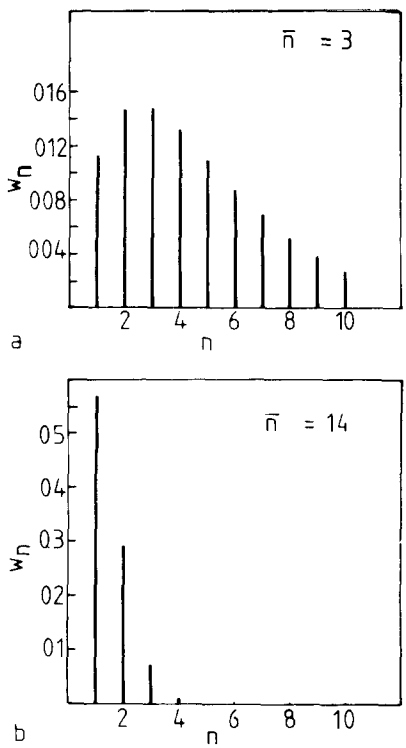

Fig. 9. Calculated weight distribution of chain length for two different values of $\bar{n}$, which both give best agreement with the experimental scattering curve, and the same values of M_w/M_n. Each of the lines in this histogram represents mean values of the chain length $\bar{n}_{is} = \bar{n} . \bar{n}_{i2}$, where $\bar{n}_{i2}$ is itself a distribution. A most probable distribution has been assumed.

Table 3 gives the corresponding molecular data, and the pair of curves (c) in Fig. 7 shows the angular dependence of $1/P_z$. An almost perfect agreement with the experiment is now obtained for two values of $\bar{n}$, i.e. $\bar{n} = 1{\cdot}4$ or $\bar{n} = 3$.

DISCUSSION

The excellent agreement of the calculated curves with the experimental angular dependence and the fact that all but one relevant parameters have not been fitted, but were rather taken from degradation experiments, give strong evidence for the validity of the modified Robin-Mercier model. There remains the uncertainty that the model allows two equivalent solutions for $\bar{n}$ (the only adjustable parameter), which is demonstrated in Fig. 10 with the polydispersity parameter. Thus, from pure model calculations we are not able to distinguish between $\bar{n} = 1{\cdot}4$ and $\bar{n} = 3$. Comparison of the GPC curves of Fig. 8 (Eschwey *et al.*, 1985) with the calculated distributions of Fig. 9 gives strong evidence for an average number of coupled chains of $\bar{n} = 1{\cdot}4$.

It might be interesting to examine the reason for the strong differences in the particle scattering factor of the three models. In previous

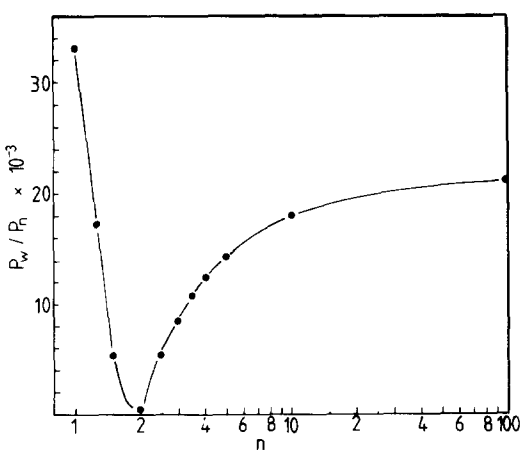

Fig. 10. Calculated polydispersities P_w/P_n as function of $\bar{n}$ (defined in Fig. 9), for the modified Robin-Mercier model. The model calculation allows two equivalent solutions of $\bar{n}$.

work it has been shown (Kajiwara *et al.*, 1970; Kajiwara, 1971; Burchard *et al.*, 1981; Burchard, 1983) that regular branching causes an upturn, while polydispersity results in a downturn, of these curves. Regular molecules are by definition monodisperse, while by random branching high polydispersities are produced (Flory, 1941, 1953; Stockmayer, 1944). In the latter, the upturn due to branching is balanced by the effect of polydispersity (Kajiwara, 1971), and a straight line is obtained.

Accordingly, the homogeneous Meyer model should exhibit lowest polydispersity, while with the introduction of structural heterogeneity broader M_w distributions are created. Comparison of the polydispersities (see Table 3), found for the different models, shows indeed an increase of polydispersity from curves (a) to (c) (Fig. 7). This point is of a certain relevance, since the question has often been discussed as to whether the interconnecting linear chains are monodisperse in length. From the arguments given here, monodispersity of the chain length can be excluded, since such structures will cause a stronger upturn in $1/P_z$ than found experimentally, though with a distribution slightly narrower than the assumed most probable length may be compatible with the experimental findings. Finally, we mention that the calculations were made with the assumption of $\bar{n}_{ic} = 2$. Though this value appears sensible for various reasons, we wish to point out that the value for $\bar{n}_{ic}$ can be determined from a careful GPC fractionation of the debranched stubs, which is possible with P2 Bio-rad gel.

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