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THE FINE STRUCTURE OF AMYLOPECTIN*

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ABSTRACT

A critical examination of an enzymic method for determining the ratio of A and B chains in amylopectin leads to a value of ~1:1, and not 2:1 as suggested by other workers. Partial debranching with pullulanase gave results consistent with earlier suggestions that A chains are predominantly and selectively removed. The ratio of A and B chains in a partially branched amylopectin has been determined, and the results are discussed in relation to possible structures for amylopectin.

INTRODUCTION

A description of the molecular structure of amylopectin and glycogen requires a knowledge of several properties, including the molecular weight, the average chain-length, the relative length of the external and internal chains, and the ratio of A and B chains.* Various models for the arrangement of the A and B chains have been proposed. In the Haworth "laminated" structure[3], the ratio of A and B chains is $1:(n-2)$ where n, the total number of chains, may be of the order of 10^4. In the Staudinger "comb" structure[4], all of the chains except one are A chains. In the original Meyer "tree-type" structure[5], and the revised Meyer structure proposed by Whelan and co-workers[6], the A:B ratio is ~1:1. The first quantitative estimate of the A:B-chain ratio resulted from studies[2,7] of the debranching of the beta-limit dextrin of waxy maize starch (amylopectin) with R-enzyme, which gave 12.8% of maltose plus maltotriose. These oligosaccharides were produced from A chains shortened by the action of beta-amylase, whereas B chains gave rise to maltohexaose and higher maltosaccharides. An A:B-chain ratio of 1:1 would produce a yield of 10.4%, so these results indicated a ratio of ~1.5:1. Since R-enzyme has no action on glycogen beta-limit dextrins, Bathgate and Manners[8] treated a number of beta-limit

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*An A chain is attached to the macromolecule by a single linkage from its potential reducing-group, whereas B chains are linked to two or more other chains[2]. The C chain is terminated by the single reducing-group in the macromolecule.

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dextrins from various amylopectins and glycogens with pullulanase and, after paper chromatography, estimated the amount of maltose plus maltotriose produced. Their results showed that the A : B-chain ratios in potato amylopectin and waxy sorghum starch were rather greater than 1 : 1, and that the ratios were of the order of 1 : 1 for seven glycogen samples.

Marshall and Whelan[9] estimated the A : B-chain ratios in a range of glycogens and amylopectins by incubating the beta-limit dextrins with isoamylase, and with isoamylase plus pullulanase. The method depends on the ability[10] of isoamylase to hydrolyse the maltotriosyl, but not the maltosyl, stubs produced by the action of beta-amylase on A chains, in conjunction with the ability of pullulanase to hydrolyse both types of stubs. Thus, pullulanase debranches all the side-chains in a beta-limit dextrin, whereas isoamylase debranches all except those that are two D-glucose residues long. The A : B-chain ratio can be calculated from the reducing-sugar equivalents produced after enzymic treatment. Using this method[9], the A : B-chain ratio of seven glycogens was found to vary between 0.6 and 1.2 : 1 in general agreement with previous results[8], but for eight amylopectins, higher values than those found previously were obtained. The lowest estimate was 1.5 : 1 for wheat amylopectin, and the highest was 2.6 : 1 for waxy sorghum and waxy maize starches. This high proportion of A chains is not in accord with the various versions of the "tree-type" molecular structure, which were based on models[6,7] containing approximately equal numbers of A and B chains, and satisfactory diagrams showing an A : B-chain ratio of ~2 : 1 have apparently not yet been produced. However, the elongated model could more readily accommodate a high ratio than could a tree-type model, where overcrowding would rapidly occur as successive tiers were formed [Fig. 1(c)].

In view of the differences of these last results[9] from those obtained by other methods, a critical examination of the determination of the ratio of A : B chains in amylopectin (waxy maize starch) has been carried out. We also describe an experiment on the partial debranching of amylopectin, which has given some information on the overall molecular structure.

EXPERIMENTAL

Beta-amylase and beta-limit dextrin. — Crystalline, sweet-potato beta-amylase was obtained from Sigma. The beta-limit dextrin obtained from waxy maize starch by using barley beta-amylase from the Wallerstein Laboratories, New York, was the sample prepared previously[11]. Beta-limit dextrins from *Helix pomatia* and rabbit-liver glycogens were also prepared previously by a similar method[8]. Soya-bean beta-amylase was prepared as described by Peat and co-workers[12].

Solutions of waxy maize starch. — Samples for the preparation of beta-limit dextrins, using soya-bean extract and crystalline, sweet-potato beta-amylase, were prepared by storing granules (500 mg) with dimethyl sulphoxide (3.0 ml) for 24 h. Acetate buffer (100mM) containing 0.02% of sodium azide was added (1 ml/10 mg),

and the mixture shaken until the starch dissolved. After dialysis against buffer, a slight precipitate that had formed was removed by centrifugation.

Beta-limit dextrins. — These were prepared by a previously published method[13], with continuous dialysis against buffer to remove maltose. In the final incubation, dialysis was against water, and the product was freeze-dried.

Determination of A : B-chain ratios. — The procedure was similar to that previously described[9], except that 2.9 nkat/ml of isoamylase (instead of 1.7 nkat/ml) was used, and the digest was diluted 5-fold before the addition of pullulanase, and centrifuged before the determination of reducing sugar.

Partly debranched, waxy maize amylopectin. — Waxy maize amylopectin (500 mg in 60 ml of 100mM acetate buffer, pH 5.0, containing 0.02% of sodium azide) was incubated with 50 nkat of pullulanase at 25°. Samples were assayed for reducing power and, when this was 32% of the maximum attainable, the reaction was stopped by boiling for 10 min. Aliquots were also assayed by gel chromatography on Sepharose 2B. The total incubation was then chromatographed in four batches on a column (3.8 × 80 cm) of Sepharose 2B, and the middle (partly included) fraction was separated from the excluded and fully included fractions. This fraction was then concentrated by using a Diaflo PM-10 membrane, and the beta-limit dextrin was prepared by using this solution.

Enzyme assays. — Alpha-amylase was assayed by using Phadebas tablets[14], α-D-glucosidase by the release of *p*-nitrophenol from *p*-nitrophenyl α-D-glucopyranoside, isoamylase by the increase in reducing power on incubation with rat-liver glycogen[15], beta-amylase (total amylolytic activity) by the increase in reducing power on incubation with waxy maize amylopectin, and pullulanase by the increase in reducing power on incubation with pullulan. Reducing power was determined by the colorimetric, Somogyi procedure[16].

Isoamylase. — Isoamylase was prepared from an extract of *Cytophaga* sp. by using DEAE-cellulose chromatography, followed by affinity chromatography on Concanavalin A-Sepharose saturated with glycogen[15]. As found previously, the final preparation caused a very slow hydrolysis of pullulan. The rate of production of reducing power from pullulan was 4.8×10^{-4} times that from rat-liver glycogen. However, this activity cannot be ascribed to a pullulanase impurity, since a slow release of maltotriose units is to be expected, because of the known ability of isoamylase to hydrolyse maltotriosyl stubs[10,15].

RESULTS AND DISCUSSION

The enzymic method for determination of A : B-chain ratios

Although the method devised by Marshall and Whelan is ingenious, it suffers from certain disadvantages which are not apparent in the detailed publication[9]. This is a consequence of the method of calculation, the procedure, and the properties of isoamylase. Firstly, the level of reproducibility is low. Thus, in determining the

production of reducing sugar, even in the best range of spectrophotometer readings, small differences can cause relatively large changes in the apparent A : B-chain ratio. For example, readings of 0.500 for isoamylase and 0.660 for both enzymes give an A : B ratio of 0.94 : 1, but if the reading for isoamylase alone should be 0.510, the A : B ratio becomes 0.83 : 1. The results reported in Table III are based on the mean value of triplicate determinations.

Secondly, the concentration of isoamylase is critical. This factor has already been noted in other work[17] using isoamylase to determine the average chain-length of glycogen. The apparent, average length of one sample was 13.9, 13.9, 12.9, 13.0, and 12.8 when isoamylase concentrations of 1.0, 4.2, 16.7, 66.7, and 266.7 nkat/ml, respectively, were used in the digests. Sufficient enzyme must be added to ensure complete debranching; in our studies, we preferred to use 33.3 nkat of isoamylase/ml instead of the 1.0 nkat/ml used in the original work[18].

During determinations of the A : B-chain ratio, if the level of isoamylase was inadvertently decreased (the purified enzyme is unstable on storage[15]), high, apparent A : B-chain ratios were obtained. Table I contains results obtained by Dr. S. Hall[19] with both glycogen and amylopectin, showing the effect of varying the concentration of isoamylase on the apparent A : B-chain ratio. The effect is most marked with amylopectin. It is clear that, if the amount of isoamylase is not adequate, A : B-chain ratios greatly in excess of 1 : 1 will be obtained. Other workers[20] have observed that isoamylase has difficulty in completely debranching amylopectin, and that branched fragments are released during enzymolysis.

By contrast, the concentration of pullulanase is not so critical. Doubling the

TABLE I

EFFECT OF VARYING THE CONCENTRATION OF ISOAMYLASE ON THE A : B-CHAIN RATIO OF BETA-LIMIT DEXTRINS

Digest No.	*Isoamylase (nkat/ml)*	*Pullulanase (nkat/ml)*	*Reducing power (as μg of glucose)*	*A : B-Chain ratio*
Amylopectin 1	0.83	—	80	4.7 : 1
2	0.83	10.0	136	
3	1.67[a]	—	90	2.5 : 1
4	1.67[a]	10.0	140	
5	3.33	—	105	1.2 : 1
6	3.33	10.0	144	
Glycogen 1	0.83	—	87	2.8 : 1
2	0.83	10.0	137	
3	1.67[a]	—	109	1.1 : 1
4	1.67[a]	10.0	147	
5	3.33	—	114	0.9 : 1
6	3.33	10.0	150	

[a]An isoamylase concentration of 1.7 nkat/ml was used in the original work[9].

amount of pullulanase caused no change in the ratios, and the recommended amount (10 nkat/ml) is substantially more than the minimum required to effect the complete debranching of any maltosyl stubs.

A third factor in the accuracy of the method relates to the apparent ability of isoamylase to hydrolyse all of the maltotriosyl stubs but none of the maltosyl stubs[10]. Incubation of amylopectin beta-limit dextrin with an isoamylase from a *Pseudomonas*[21] released maltose and maltotriose in a molar ratio of 1 : 13, and from glycogen beta-limit dextrin in a ratio of 1 : 10. More recently, we have detected[15] the presence of maltose in digests containing a purified *Cytophaga* isoamylase and amylopectin beta-limit dextrin, or maltosyl-maltosaccharide alpha-limit dextrins. Moreover, the rate of hydrolysis of 6^3-α-maltotriosylmaltotriose was relatively low. This partial release of maltosyl side-chains and the possible incomplete hydrolysis of maltotriosyl side-chains may be an additional limitation in the method.

The fine structure of beta-limit dextrins

A possible reason for the different values obtained for A : B-chain ratios could be the purity of the beta-amylase used in the preparation of the beta-limit dextrins. Traces of alpha-amylase or α-D-glucosidase could act on the maltosyl and maltotriosyl A-chain stubs in a beta-limit dextrin to varying extents, to give stubs with single D-glucose residues. These stubs would not be debranched by either isoamylase or pullulanase in the overall, enzymic debranching, and hence production of reducing sugar would be incomplete. Beta-limit dextrins were therefore obtained or prepared by using different beta-amylase preparations, as follows: (*a*) ungerminated barley, (*b*) soya-bean extract heated both with and without EDTA to destroy alpha-amylase[12], (*c*) crystalline, sweet-potato beta-amylase, and (*d*) crystalline, sweet-potato beta-amylase chromatographed to remove α-D-glucosidase[22].

The apparent alpha-amylolytic activity in these preparations was examined by using a dyed amylopectin substrate[14], and α-D-glucosidase activity using *p*-nitrophenyl α-D-glucopyranoside. The results are shown in Table II. All of the samples of beta-amylase contained some apparent alpha-amylase activity, but levels of activity of

TABLE II

RELATIVE LEVELS OF APPARENT ALPHA-AMYLASE AND α-D-GLUCOSIDASE IN BETA-AMYLASE PREPARATIONS

Enzyme preparation	*Ratio of alpha-amylase to total amylolytic activity*	*Ratio of α-D-glucosidase to total amylolytic activity*
Ungerminated barley	1.5×10^{-2}	$3.7 \cdot 10^{-1}$
Soya-bean extract heated with EDTA	6.0×10^{-5}	1.9×10^{-1}
Soya-bean extract heated in the absence of EDTA	5.7×10^{-5}	2.4×10^{-4}
Crystalline sweet-potato	6.3×10^{-6}	6.2×10^{-4}
Crystalline sweet-potato after chromatography	3.0×10^{-7}	Not detectable

TABLE III

THE A : B-CHAIN RATIO IN VARIOUS BETA-LIMIT DEXTRINS

Source of α-D-glucan	*Enzyme preparation used to prepare beta-limit dextrin*	*A : B-Chain ratio (± 0.1)*
Waxy maize starch	Soya bean (heated)	1.0
Waxy maize starch	Barley	0.9
Waxy maize starch	Crystalline sweet-potato	1.0
Waxy maize starch	Purified, crystalline sweet-potato	0.9
Helix pomatia glycogen	Barley	0.6
Rabbit-liver glycogen	Barley	0.8

10^{-6} and 10^{-7} of the total activity did not cause significant, random fragmentation of the amylopectin molecule. EDTA had little or no effect on the alpha-amylase activity of the soya-bean extracts. All of the beta-amylase preparations, except one, contained minute traces of α-D-glucosidase activity.

In the preparation of beta-limit dextrins, the sample using the barley extract was that previously prepared[11]. For this, waxy maize starch had been dissolved in alkali followed by neutralisation. The limit dextrin was prepared by incubation without dialysis and the product isolated by precipitation into ethanol. The glycogen samples were also previously prepared samples[8]. With the other three preparations, the amylopectin was dissolved by shaking in cold buffer after pre-treatment with dimethyl sulphoxide, which was removed by dialysis. During incubation with beta-amylase, the solution was dialysed and the product isolated by freeze-drying. The beta-limit dextrins were compared by chromatography on Sepharose 2B, and there were significant variations. The two crystalline, sweet-potato preparations gave products that were mostly in the excluded fraction with a lesser included-fraction, but the dextrins from soya-bean and barley were mostly included with a smaller excluded fraction, indicating a lower molecular weight.

These samples and two glycogen beta-limit dextrins were then treated with isoamylase and isoamylase plus pullulanase. The procedure was similar to that previously described[9], but the concentration of isoamylase was higher. The results are shown in Table III. With all preparations of the beta-limit dextrins of waxy maize amylopectin, the A : B-chain ratios were virtually 1 : 1 and showed no significant differences between samples prepared by using the various beta-amylases. These results differ from those obtained by Marshall and Whelan[9], but are in better agreement with A : B-chain ratios determined by the estimation of maltose plus maltotriose after debranching by pullulanase[8]. The glycogen ratios are lower than those for amylopectin and are in general agreement with previously determined values[8,9]. We conclude that the difference in our results for waxy maize starch and those reported by Marshall and Whelan[9] is not due to subtle structural variations in the beta-limit dextrins.

The molecular structure of amylopectin

A number of different, possible arrangements of A and B chains in model structures of the amylopectin molecule have been suggested[3-6]. Two extreme models are the revised, Meyer spherical structure[6] in which some B chains are multiply branched with other B chains, and the very extended, Haworth "laminated" structure[3], although the latter is now ruled out by the results for the A:B-chain ratio. More recently, Whelan and co-workers[23,24] have proposed an alternative model structure for amylopectin which combines some of the features of the Haworth "laminated"[3] and Staudinger "comb" structure[4] (Fig. 1). As originally drawn[23], this structure is in accord with an A:B-chain ratio of 1:1 and, being asymmetrical, has the advantage of explaining some of the differences in physical properties between amylopectin and glycogen, notably its higher viscosity and behaviour on ultracentrifugation. In an attempt to distinguish between the multiply branched, tree-like structure, or modifications thereof[25,26], and the new, elongated model[23,24], the debranching of waxy maize amylopectin with pullulanase was studied, and the A:B-chain ratio of the partly debranched material was determined.

Pullulanase has an endo action on pullulan, as shown by a comparison of the reducing power and maltotriose released[27], viscosity changes *versus* reducing power[28], and chromatographic examination of the hydrolysis products[27,28]. On the other hand, present evidence suggests that the debranching of amylopectin is not an endo action, but that pullulanase predominantly hydrolyses the outer inter-chain linkages and, at intermediate stages of hydrolysis, scarcely affects the innermost inter-chain linkages. From an examination on Sephadex G-75 of the products of partial de-

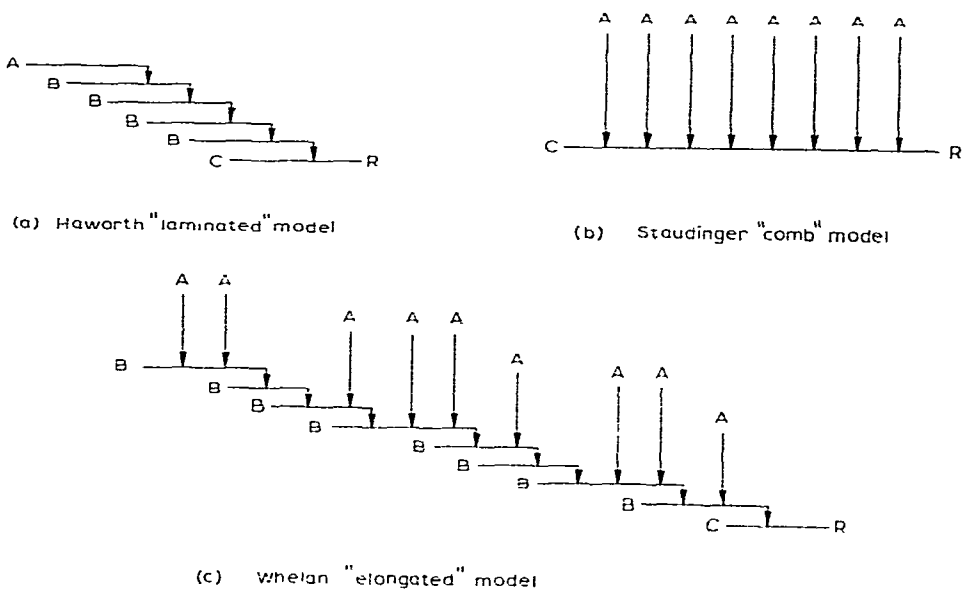

Fig. 1. Structural models of amylopectin. Key: A, B, and C denote various types of chain; R is the potential reducing-group.

branching of amylopectin by pullulanase and by isoamylase, Harada and co-workers[29] concluded that pullulanase selectively hydrolyses the outermost inter-chain linkages. A similar conclusion was reached by Atwell *et al.*[20] from a study of pullulanase action on wheat amylopectin.

In the present study, waxy maize starch was slowly debranched with pullulanase (10 mg of starch and 0.6 nkat of pullulanase per ml) and the process was monitored by gel chromatography on Sepharose 2B. On this support, the original, waxy maize starch was excluded and the completely debranched product was fully included. Fig. 2 shows the pattern obtained when 32% of maximal debranching had occurred and indicates the appearance of partially debranched polysaccharide as a partly included fraction. When the debranching was 14% of the maximum (Table IV), there was a similar percentage of the total glucan as fully included fraction, and the absorption spectrum of this with iodine was the same as that of completely debranched glucan, *i.e.*, the λ_{max} was higher than for amylopectin. The result suggests that, after 14% of debranching, the main reaction has been the release of A chains. When 32 and 42% of debranching had occurred, the iodine complex of the fully included material still showed a high λ_{max}, and the amounts of polysaccharide were similar to the percentage debranching. The K_{av}* of the partly included material indicated

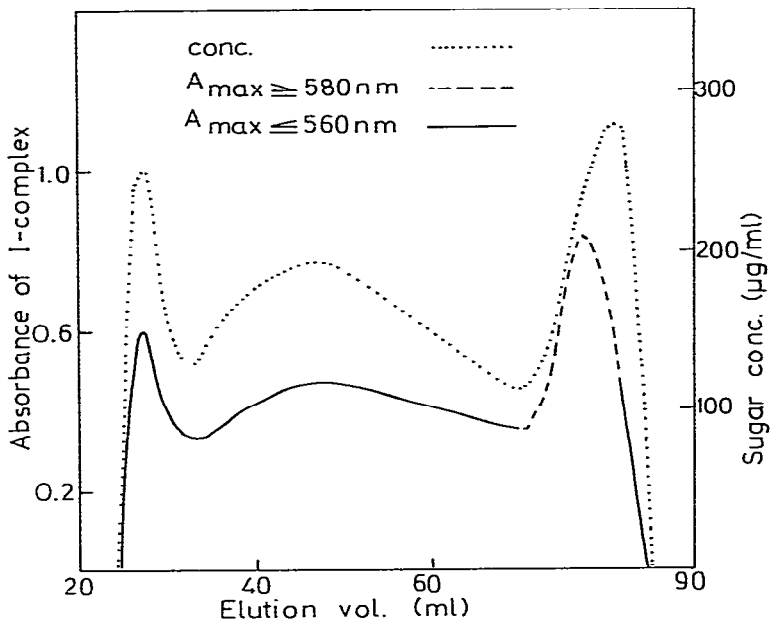

Fig. 2. Gel chromatography on Sepharose 2B of waxy maize starch after partial debranching by pullulanase. Key: ·····, carbohydrate (determined by the phenol–sulphuric acid reagent); ——, polysaccharide with an iodine complex having λ_{max} at 560 nm; – – –, carbohydrate with an iodine complex having λ_{max} at 580 nm.

*$K_{av} = \frac{\text{elution volume} - \text{void volume}}{\text{total volume} - \text{void volume}}$.

TABLE IV

PARTIAL HYDROLYSIS OF WAXY MAIZE AMYLOPECTIN WITH PULLULANASE

Percent of maximal debranching	*Amounts of glucan in fractions from Sepharose 2B (% of total)*			*K_{av} of partly included fraction*
	Excluded	*Partly included*	*Fully included*	
0	100	0	0	—
14	33	52	15	—
32	14	59	27	0.35
42	3	58	39	0.51
60	1	46	53	0.60
100[a]	0	0	100	—

[a]Determined in a separate experiment with excess of pullulanase.

that it was of high molecular weight. At 32 and 42% of maximal debranching, the material had K_{av} values similar to those of standard dextran preparations having molecular weights of 2×10^6 and 7×10^5, respectively. On Sepharose 6B, chromatography of another partial hydrolysate with 35% of debranching showed mainly excluded and fully included material. Even when 60% of debranching had occurred, the iodine spectrum of the fully included material was still similar to that of unbranched chains and there was a partly included fraction.

The results are in agreement with those of Atwell *et al.*[20] and Harada *et al.*[29], which indicated that pullulanase debranches amylopectin by predominantly hydrolysing A chains. This is more consistent with a relatively compact, but asymmetrical, "tree-like" structure for amylopectin, rather than the more-open, elongated structure[23,24]. In the former, steric hindrance to hydrolysis of the interior inter-chain linkages would be more likely.

A sample that had undergone 35% of maximum debranching was fractionated on Sepharose 2B on a preparative scale and the partly included fraction collected. On re-chromatography, it showed a single peak. It was then converted into the beta-limit dextrin and the A : B-chain ratio was estimated with pullulanase and isoamylase.

If the original amylopectin had the elongated structure, hydrolysis of A chains would leave all the B chains as B chains, except for the terminal B chains, which would become A chains. If 35% of debranching occurred in a polysaccharide having an original A : B-chain ratio of 1 : 1, the remaining polymer would have a ratio of

$$\frac{\text{fraction of original A chains} - 0.35}{\text{fraction of original B chains}} = \frac{0.50 - 0.35}{0.50} = 0.3 : 1.$$

If the original polymer had a tree-like or modified tree-like structure, removal of a proportion of the A chains would convert some of the B chains into A chains, but leave others as B chains. The net result would be a partly debranched polymer

having a lower A:B-chain ratio than the original polysaccharide, but a higher A:B-chain ratio than that calculated for the elongated model. The magnitude of the change in the ratio cannot be calculated, since it depends on the precise arrangement of the A and B chains. For example, a B chain linked to two A chains would remain a B chain if one of the two A chains were removed, but might itself become an A chain if both A chains were hydrolysed.

When parallel estimations of the A:B-chain ratios were performed with the non-branched, and partly debranched, beta-limit dextrins, for which the original amylopectin had a ratio of $1.0(\pm 0.1):1$, the value obtained for the partly debranched material was $0.6(\pm 0.1):1$. This limited lowering of the A:B-chain ratio is considered to be more consistent with a tree-like or modified tree-like structure than with the elongated model.

Although many of the experimental results for amylopectin are in accord with a Meyer tree-like structure, we now believe that a modification of this structure, namely, a cluster-type structure, is more probable (Fig. 3). A cluster-type structure for a "starch molecule" was originally proposed by Nikuni[30] and, independently, for the amylopectin component by French[25], and later by Robin and co-workers[26]. More recently, an extended cluster model has been proposed by French and co-workers[31] on the basis of electron microscopic studies on waxy maize starch. A structure of the cluster-type is more in accord with the physical properties, the observed structural features, and with its mode of biosynthesis within the starch granule. For example, there is good evidence from other enzymic degradation studies[32,33] that the multiply branched amylopectin structure contains branch points that are arranged in tiers of some kind, a feature which is missing from the more-elongated model[23,24].

In conclusion, we wish to emphasise one major and inherent difficulty in all methods for determining the fine structure of amylopectin. A typical molecule may

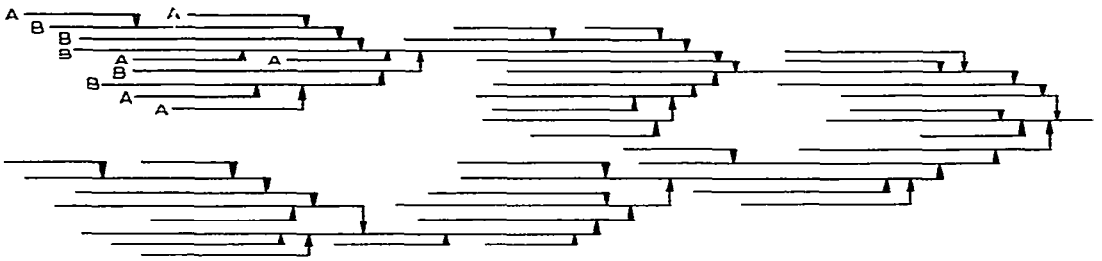

Fig. 3. Cluster model of amylopectin, based on French[25] and Robin *et al.*[26]. The various A and B chains, which are shown as straight lines, may actually exist in double-helix conformations. The model expresses the concept of "clusters" of inter-chain linkages within certain regions of the macromolecule, and is not intended to be a complete representation of an amylopectin molecule, which may comprise 10^4–10^5 individual chains. A proportion of the B chains must carry more than one A-chain, to account for the change in the A:B-chain ratio of the partly debranched material. This feature is also required in the revised Meyer structure[6] and the elongated model[23,24]. The inter-chain linkages are denoted by the vertical arrows, and R represents the sole, potential reducing-group in the molecule.

contain about 10,000 constituent chains and there can be no guarantee that all of the exterior chains are degraded to the full extent during the preparation of limit dextrins. Even using crystalline and highly purified enzymes, the successive action of muscle phosphorylase and beta-amylase on amylopectin gave[10] a phi,beta-dextrin that still had a beta-amylolysis limit[6] of 5%. There is also the possibility that some chains may terminate in the interior of the molecule ("buried" chains) and be relatively inaccessible to starch-degrading enzymes acting in an exo-manner[25]. It is therefore obvious that, despite every precaution, the degradation of some exterior chains may not be complete, and that this will lower the quantitative nature of any experimental measurements, and hence decrease the significance of any deductions which are drawn therefrom. The present paper should therefore be regarded as an interim report rather than a definitive solution of a continuing investigation on the fine structure of amylopectin.

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REFERENCES

1 R. S. Hall and D. J. Manners, *Carbohydr. Res.*, 83 (1980) 93–101.
2 S. Peat, W. J. Whelan, and G. J. Thomas, *J. Chem. Soc.*, (1952) 4546–4548.
3 W. N. Haworth, E. L. Hirst, and F. A. Isherwood, *J. Chem. Soc.*, (1937) 577–581.
4 H. Staudinger and E. Husemann, *Justus Liebigs Ann. Chem.* 527 (1937) 195–236.
5 K. H. Meyer, *Experientia*, 8 (1952) 405–420.
6 Z. Gunja-Smith, J. J. Marshall, C. Mercier, E. E. Smith, and W. J. Whelan, *FEBS Lett.*, 12 (1970) 101–104.
7 S. Peat, W. J. Whelan, and G. J. Thomas, *J. Chem. Soc.*, (1956) 3025–3030.
8 G. N. Bathgate and D. J. Manners, *Biochem. J.*, 101 (1966) 3c–5c.
9 J. J. Marshall and W. J. Whelan, *Arch. Biochem. Biophys.*, 161 (1974) 234–238.
10 Z. Gunja-Smith, J. J. Marshall, E. E. Smith, and W. J. Whelan, *FEBS Lett.*, 12 (1970) 96–100.
11 O. Kjolberg, Ph.D. Thesis, University of Edinburgh, 1962.
12 S. Peat, S. J. Pirt, and W. J. Whelan, *J. Chem. Soc.*, (1952) 714–722.
13 W. J. Whelan, *Methods Carbohydr. Chem.*, 4 (1964) 261–266.
14 M. Ceska, K. Birath, and B. Brown, *Clin. Chim. Acta*, 26 (1969) 437–444.
15 R. M. Evans, D. J. Manners, and J. R. Stark, *Carbohydr. Res.*, 76 (1979) 203–213.
16 J. F. Robyt and W. J. Whelan, in J. A. Radley (Ed.), *Starch and its Derivatives*, Chapman and Hall, London, 4th edn., 1968, pp. 432–433.
17 R. M. Evans, unpublished work.
18 Z. Gunja-Smith, J. J. Marshall, and E. E. Smith, *FEBS Lett.*, 13 (1971) 309–311.
19 R. S. Hall, Ph.D. Thesis, Heriot-Watt University, 1978.
20 W. A. Atwell, R. C. Hoseney, and D. R. Lineback, *Cereal Chem.*, 57 (1980) 12–16.
21 K. Yokobayashi, A. Misaki, and T. Harada, *Biochim. Biophys. Acta*, 212 (1970) 458–469.
22 J. J. Marshall and W. J. Whelan, *Anal. Biochem.*, 52 (1973) 642–646.
23 W. J. Whelan, *J. Jpn. Soc. Starch Sci.*, 23 (1976) 101–115.
24 D. Borovsky, E. E. Smith, W. J. Whelan, D. French, and S. Kikumoto, *Arch. Biochem. Biophys.*, 198 (1979) 627–631.
25 D. French, *J. Jpn. Soc. Starch Sci.*, 19 (1972) 8–25.

26 J. P. ROBIN, C. MERCIER, R. CHARBONNIERE, AND A. GUILBOT, *Cereal Chem.*, 51 (1974) 389–406.
27 G. S. DRUMMOND, E. E. SMITH, W. J. WHELAN, AND HAN TAI, *FEBS Lett.*, 5 (1969) 85–88.
28 R. OHBA AND S. UEDA, *Agric. Biol. Chem.*, 39 (1975) 967–972.
29 T. HARADA, A. MISAKI, H. AKAI, K. YOKOBAYASHI, AND K. SUGIMOTO, *Biochim. Biophys. Acta*, 268 (1972) 497–505.
30 Z. NIKUNI, *Chōri-Kagaku*, 2 (1969) 6–14; *J. Jpn. Soc. Starch Sci.*, 22 (1975) 78–92.
31 M. YAMAGUCHI, K. KAINUMA, AND D. FRENCH, *J. Ultrastruct. Res.*, 69 (1979) 249–261.
32 J. LARNER, B. ILLINGWORTH, C. T. CORI, AND C. F. CORI, *J. Biol. Chem.*, 199 (1952) 641–651.
33 B. S. ENEVOLDSEN AND F. SCHMIDT, *J. Inst. Brew., London*, 80 (1974) 520–533.