A REVISION OF THE MEYER-BERNFELD MODEL OF GLYCOGEN AND AMYLOPECTIN

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1. Introduction

In the period before it became possible to explore the fine structures of glycogen and amylopectin with enzymes, several structures were proposed that equally satisfied the then known facts. The discovery of debranching enzymes specifically hydrolysing the 1→6-branch points in the polysaccharides made it possible to distinguish between the alternatives. The regularly rebranched structure originally proposed by Meyer and Bernfeld [1] was the only one of the then existing proposals that could fit the results of exploration of glycogen [2] and amylopectin [3] with debranching enzymes, and the structure shown in fig. 1 has been that generally accepted.

The enzymic investigations were basically concerned with determining the relative number of *A* and *B* chains in the branched molecules. An *A* chain is defined as one connected to the remainder of the molecule only through its reducing chain end. A *B* chain is also joined in this way but carries other *A* and/or *B* chains at one or more of its primary hydroxyl groups. Every molecule is supposed to contain a single *C* chain, unsubstituted at its reducing end. The enzymic analyses gave *A*:*B* chain ratios of about unity, fitting the Meyer model.

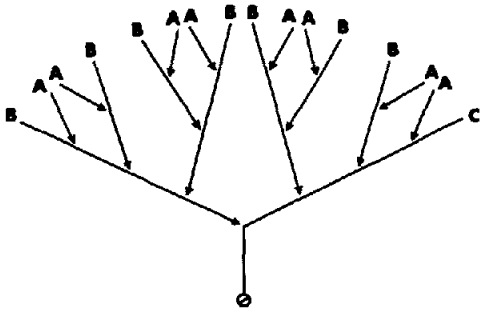

Fig. 1. Glycogen or amylopectin drawn as a regularly rebranched structure [1]. For definition of *A*, *B* and *C* chains see the text. ϕ = Reducing chain end.

The usual way in which the Meyer structure is drawn (fig. 1) is, as French [4] has pointed out, only one of many ways in which the *A*:*B*=1 condition can be satisfied. It has not, however, been possible hitherto to distinguish between variants of the Meyer structure. This we have now been able to do with a debranching enzyme (isoamylase) described in the accompanying paper [6]. The special features of this enzyme that have allowed us to explore the problem in question are (i) isoamylase totally debranches glycogen and amylopectin, permitting an inspection of the lengths of the unit chains in these molecules, and (ii) it does not remove 1→6-bonded α-maltosyl units. We can use two enzymes, phosphorylase and β-amylase, to create these isoamylase-resistant units in the macromolecules and therefore "label" certain structural features. These experiments prove that the *A* and *B* chains of both glycogen and amylopectin are not arranged as in the regularly rebranched structure. A model more compatible with the facts is proposed.

2. Experimental

ϕ, β-Dextrins of shellfish glycogen and amylopectin were prepared by treatment of the corresponding muscle phosphorylase limit dextrins [5] (15 mg/ml) with sweet potato β-amylase (100 U/ml) in 10 mM acetate buffer pH 5.0 for 24 hr at 37°. Maltose was then removed by dialysis against water. All other

Table 1
Actions of isoamylase and isoamylase + pullulanase on glycogen and amylopectin ϕ, β-dextrins.

ϕ, β-Dextrin	Glucosidic bonds hydrolysed (%)		Degree of β-amylolysis (%) after *:	
	Isoamylase	Isoamylase + pullulanase	Isoamylase	Isoamylase + pullulanase
Shellfish glycogen	8.7	16.4	44	97
Waxy maize amylopectin	4.8	11.2	29	99

* The degrees of β-amylolysis of the ϕ, β-dextrins before debranching were: glycogen, 2.5%; amylopectin, 5%.

materials and methods were as described in the preceding paper [6].

3. Results and discussion

3.1. *The fine structure of glycogen*

A single simple experiment serves to demonstrate the incorrectness of the ideal Meyer structure (fig. 1) as it applies to glycogen. This makes use of what may by called glycogen ϕ, β-dextrin. This is glycogen degraded successively with muscle phosphorylase and β-amylase. By this means the *A* chains are reduced uniformly to 2 units (maltose) in length [7]. It is a property of *Cytophaga* isoamylase that it will not hydrolyse 1→6-bonded α-maltosyl residues [6]. Therefore, no *A* chains will be removed from the ϕ, β-dextrin by isoamylase. The *B*→*B* branch linkages will be hydrolysed. Inspection of the ideal Meyer structure (fig. 1) shows that every *B* chain liberated by isoamylase from the Meyer ϕ, β-dextrin would still be terminated near its non-reducing end by a maltosyl *A* chain, exactly as it was in the undebranched molecule. *Therefore the debranched molecule should remain inert to the action of β-amylase.* We find (table 1) that, as expected, isoamylase hydrolyses only half the number of branch points of shellfish glycogen ϕ, β-dextrin that are hydrolysed by a mixture of isoamylase and pullulanase (table 1). (Pullulanase, unlike isoamylase, removes the maltosyl *A* chains [8].) A chromatogram of the isoamylase digest (fig. 2A), compared with the isoamylase-pullulanase digest (fig. 2B), confirms the absence of maltose from the former. However, the degree of β-amylolysis of isoamylase-treated ϕ, β-dextrin is far from being nil. It is 44% (table 1). This result tells us either that many *B* chains do not carry an *A* chain, or, if they do, the *A* chain is not available to phosphorylase and β-amylase. Perhaps both possibilities are true. The second possibility, the occurrence of what French [4] has called "buried chains", deserves serious consideration. It is easy to conceive that a chain whose non-reducing terminus lies within the interior of the giant macromolecule may be inaccessible to enzymes such as phosphorylase and β-amylase, themselves having molecular weights of about 200,000. We do not think, however, that buried chains alone can account for the results in table 1, simply because of the magnitude of the attack of β-amylase on isoamylase-treated ϕ, β-dextrin.

Isoamylase has enabled us for the first time to observe the profile of unit chains in glycogen. Fig. 3 shows the results of debranching rabbit-liver glycogen with isoamylase and fractionating the products on Sephadex G-50. There is seen a symmetrical weight distribution of chains around an average length of 14, which is in fact the overall average length for the glycogen. It will be appreciated that the distribution in terms of *numbers* of chains is unsymmetrical, there being a preponderance of smaller chains, the numbers falling away as the chains length increases. This is precisely what is predicted by the Meyer structure.

In explanation of the combined observations we offer the structure shown in fig. 4. This is intended only to express certain concepts and is not to be regarded as precisely defining glycogen structure. In this model half the *B* chains carry an average of 2 *A* chains, while the other *B* chains each carry 2 *B* chains. The model has been drawn in its most symmetrical

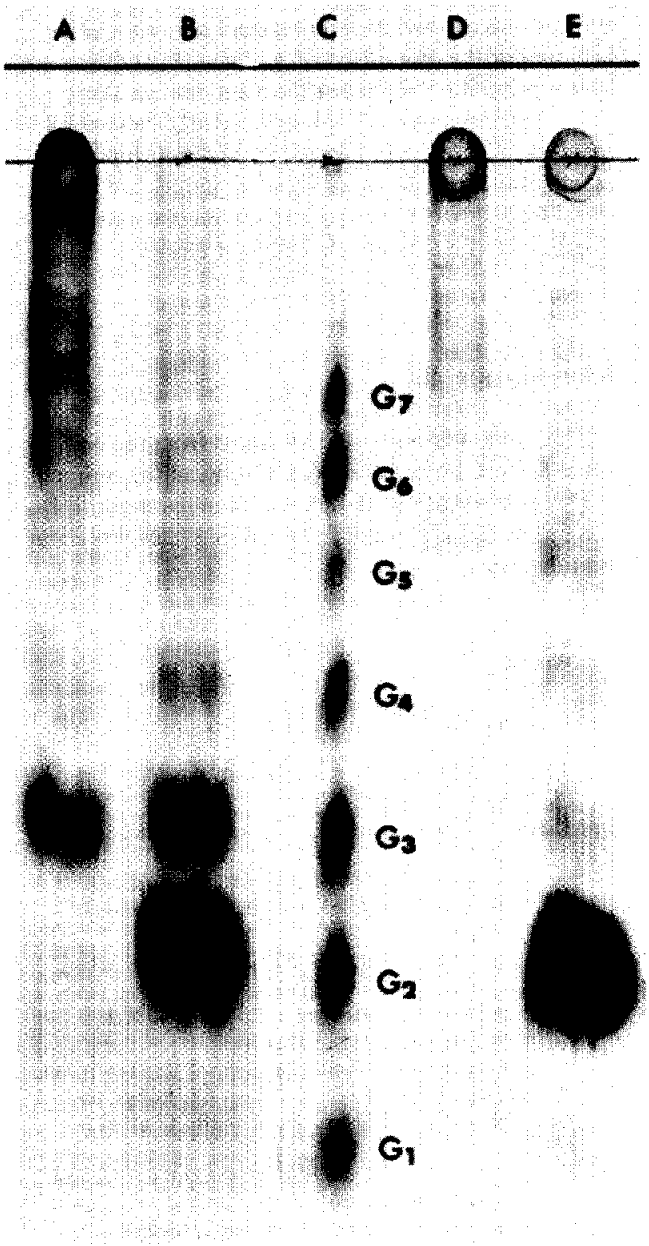

Fig. 2. Chromatography of products of debranching of shellfish glycogen and amylopectin ϕ, β-dextrins. A and B are the products of the action of purified isoamylase and pullulanase respectively on glycogen ϕ, β-dextrin. D and E show the corresponding products from amylopectin ϕ, β-dextrin. C is glucose (G1) and maltodextrin (G2, G3 etc.) standards. The digest conditions and method of chromatography were as described in the preceding paper [6].

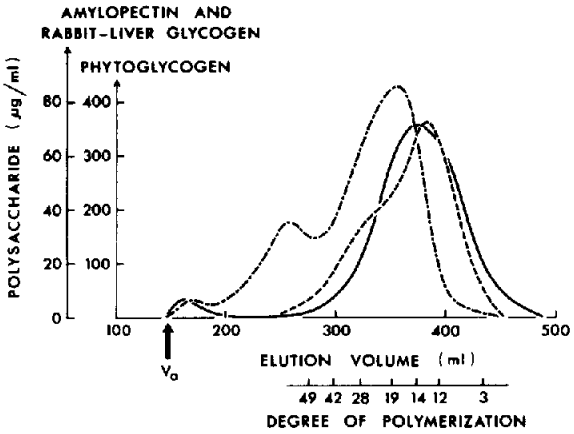

Fig. 3. Sephadex G-50 fractionation of the products of isoamylase-debranching of rabbit liver glycogen (—), phytoglycogen (- - -) and amylopectin (- · -). The polysaccharides (50, 250 and 30 mg respectively) were debranched using 0.5–1.0 mg of crude isoamylase [6] per mg of polysaccharide in 25–40 mM acetate buffer pH 5.5 at 37°. When debranching was complete (24–48 hr, as measured by degree of β-amylolysis [7]) the enzyme was inactivated, insoluble material removed by centrifugation, and samples of the digests containing 10–30 mg of polyglucose were fractionated as described elsewhere [10].

form but we would envisage variations from this symmetry in the actual polymer, both in regard to chain length and average degree of subsitution of the chains.

The model explains the susceptibility of isoamylase-treated ϕ, β-dextrin to β-amylase. It also contains what may be regarded as buried *B* chains, though buried *A* chains could be drawn in. The concept of the buried chain arises naturally out of the consideration that it is unlikely that every chain in the molecule always

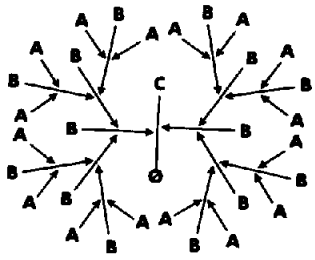

Fig. 4. A schematic representation of glycogen or amylopectin. The symbolism is as in fig. 1, but the arrangement of chains differs in two ways. Only half the *B* chains carry *A* chains, and half the *B* chains have their non-reducing termini inside the molecule and not at the surface.

has an equal chance of further growth. The Meyer formula (fig. 1) assumes equal growth and that all chains have their non-reducing ends at the surface of the molecule.

Phytoglycogen was also debranched with isoamylase and fractionated (fig. 3). Though this glycogen has hitherto been regarded as very similar to the animal variety, fig. 3 shows that the unit-chain profile is distinctly different from that of liver glycogen. Whether this means that the arrangement of chains in phytoglycogen is different from liver glycogen remains to be determined.

3.2. *The fine structure of amylopectin*

Action of isoamylase on amylopectin ϕ, β-dextrin did not liberate maltose, but pullulanase did liberate this sugar (fig. 2D, E). The isoamylase-treated dextrin yielded 29% maltose with β-amylase (table 1). Therefore the Meyer structure can again be discounted. The unit-chain profile of isoamylase debranched amylopectin is shown in fig. 3. There are seen two peaks, one of average length about 20 and the other in excess of 50. This asymmetry mirrors that of phytoglycogen, and one may speculate on the possibility of a fundamental difference both in structure and method of biosynthesis of the two plant polysaccharides, as opposed to liver glycogen.

In an earlier paper [9] we showed the unit-chain profile of amylopectin after debranching with pullulanase. In this fractionation there was seen a prominent third peak excluded from the gel. We now know that this material is branched and resistant to pullulanase. It is however debranched by isoamylase, and low molecular weight oligosaccharides are liberated. We conclude that amylopectin contains either glycogen-like areas or even a separate population of molecules that are as highly branched as glycogen, the allusion to glycogen being made on the basis that the material is pullulanase-resistant but isoamylase susceptible, as is rabbit liver glycogen.

In conclusion we may point out that a comparison of the extents of hydrolysis of ϕ, β-dextrin by isoamylase and isoamylase + pullulanase permits a determination of the proportion of *A* chains. In addition, we have separated on Sephadex the β-amylase resistant chains from isoamylase-treated β-dextrin. These are *B* chains terminated with maltose stubs. Their further examination after debranching with pullulanase will reveal for the first time what are the precise inner lengths of those *B* chains that carry *A* chains.

Acknowledgements

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