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SPATIAL DISTRIBUTION OF UNIT CHAINS IN GLYCOGEN

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ABSTRACT

Cytophaga isoamylase is shown to catalyse an ordered and sequential exo-hydrolysis of the (1→6)-α linkages in glycogen, cleaving component (1→4)-α-D-glucosyl chains from the macromolecule in order of increasing depth of (1→6)-α linkage to the polymer. The resulting pattern of maltodextrin release by isoamylase as a function of the degree of debranching consequently defines the spatial arrangement of chains in the macromolecule. Studies showed consistently that the degree of polymerisation of the maltodextrin products of isoamylolysis of oyster glycogen increased as a direct function of the degree of debranching. Similarly, the chain length of the polysaccharide products of partial isoamylolysis apparently increased as debranching proceeded. These results are argued to imply that glycogen has a spherical Meyer-type structure.

INTRODUCTION

The structure of glycogen has been examined by chemical and enzymic methods[1], and various structural models have been proposed[1–4]. The polysaccharide consists of (1→4)-α-D-glucosyl chains interconnected by (1→6)-α linkages**. Isoamylase (amylopectin 6-glucanohydrolase, EC 3.2.1.68) has proved an invaluable tool in structural analysis, in that it quantitatively hydrolyses the (1→6)-α linkages in glycogen[5,6]. Gel filtration of the maltodextrin products of isoamylolysis therefore allows the distribution of component chains in the polysaccharide to be defined[3,7]. There is one outstanding problem and that relates to the intramolecular organisation of (1→4)-α-D-glucosyl chains as a function of their length ($\overline{\text{d.p.}}$). Is the spatial distribu-

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**Three types of glucosyl chain may be delineated: A chains that are connected to the remainder of the molecule only through the reducing chain-end; B chains that are also joined in this way, but carry other A and/or B chains at one or more of the primary hydroxyl groups; C chains (one per molecule) that are either unsubstituted at the reducing end or attached to a protein primer-molecule[8].

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tion of component chains dependent or independent of $\overline{d.p.}$? The purpose of this study was to use the exo-specificity of *Cytophaga* isoamylase to investigate the spatial distribution of chains in the glycogen macromolecule.

RESULTS AND DISCUSSION

Action pattern of Cytophaga *isoamylase on glycogen.* — Harada *et al.*[9] reported that *Pseudomonas* isoamylase is exo-acting with glycogen (oyster) as substrate. It is now apparent that *Cytophaga* isoamylase acts in an analogous fashion. Partial isoamylolysates of oyster glycogen (see Experimental) corresponding to 21, 34, 41, 53, 61, 74, and 96% debranching were chromatographed on Bio-Gel P-10. Native oyster-glycogen was eluted in the void volume of the column (120–130 mL), whereas chromatography of 100% debranched glycogen yielded the type of elution profiles encompassing maltodextrins of $\overline{d.p.}$ 3–50 described elsewhere[3,7]. At all intermediary degrees of debranching, gel filtration produced two distinct polyglucose peaks, I and II. Typical elution profiles are shown in Fig. 1. Peak I was eluted in the void volume and contained polysaccharide material that did not react with Nelson's reagent. The size of peak II (elution volume, 380 mL; equivalent to $\overline{d.p.}$ ~7) increased as isoamylolysis proceeded. This increase was accompanied by a parallel and reciprocal decrease in the polysaccharide peak.

Treatment (24 h, 37°, pH 5.0) of column fractions from peak II with isoamylase (final concentration, 0.5 U/mL) and/or pullulanase (final concentration, 2 U/mL) resulted in no increase in reducing end-groups. An increase would be expected if branched oligosaccharides were present: isoamylase attacks (1→6)-α-linked maltodextrinyl chains, except 6-α-maltosyl groups which are acted upon by pullulanase[6,10,11]. The implication is that peak II is composed of linear maltodextrins. However, small increases in reducing end-groups (<5%) would not necessarily be detected, and corroboratory evidence was therefore sought.

Partial isoamylolysates of oyster glycogen corresponding to 0, 13, 27, 31, 73,

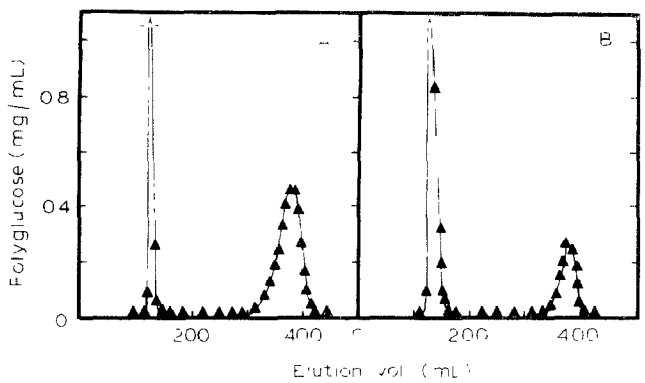

Fig. 1. Typical elution profiles on Bio-Gel P-10 of the products of partial isoamylolysis of oyster glycogen; samples corresponded to 61% (A) and 41% (B) debranching

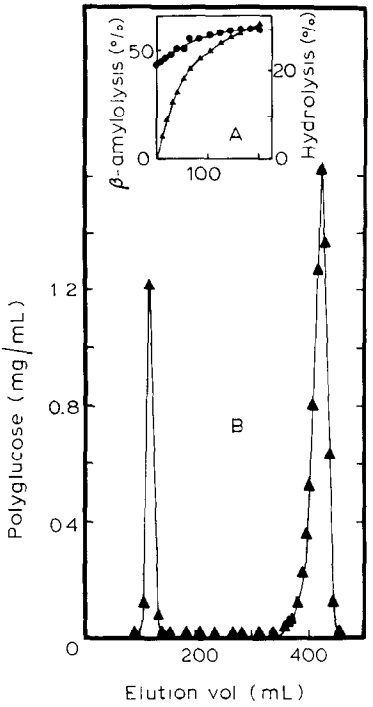

Fig. 2. The action of beta-amylase on partial isoamylolysates of oyster glycogen: A, time course of isoamylolysis (%, —▲—) and beta-amylolysis (%, —●—); B, elution profile on Bio-Gel P-10 of a beta-amylolysate (degree of beta-amylolysis, 61%) of 31% debranched glycogen.

96, and 100% debranching were treated exhaustively with beta-amylase. Consistent with an exo-mode of action, the degree of beta-amylolysis (%) of partial isoamylolysates increased in parallel with debranching (%) (Fig. 2A). The beta-amylolysates were chromatographed on Bio-Gel P-10; a typical elution-profile is shown in Fig. 2B. There were consistently two distinct peaks; one comprised polysaccharide material eluted in the void volume, and the second (elution volume, 425 mL) contained oligosaccharides of low molecular weight. This second peak ($\overline{d.p.}$ 2.1) was confirmed by gel filtration[12] on Bio-Gel P-2 and by paper chromatography to contain only maltose and maltotriose. Treatment (24 h, 37°, pH 5) of column fractions containing oligosaccharides with isoamylase (final concentration, 0.5 U/mL) and/or pullulanase (final concentration, 2 U/mL) produced no increase in reducing end-groups. The combination of gel filtration on Bio-Gel P-10 and P-2, paper chromatography, and enzymic debranching would have detected small quantities of branched oligosaccharides (<1% of total products). It is therefore inferred that branched oligosaccharides are not produced in measurable amounts by isoamylase action on glycogen.

Given that linear maltodextrins appear to be the exclusive products of isoamylase action on oyster glycogen, it is concluded that, like *Pseudomonas* isoamylase[9], *Cytophaga* isoamylase is exo-acting with glycogen as substrate, *i.e.*, the enzyme

catalyses an ordered and sequential hydrolysis of (1→6)-α linkages proceeding inwards from the non-reducing chain-ends. Enzyme action on rabbit-liver and bovine-liver glycogens is similarly exo: gel filtration on Bio-Gel P-10 of partial isoamylolysates of these polysaccharides showed linear maltodextrins to be the preponderant, if not exclusive, reaction products. It must be emphasised that this exo-specificity is a function of polysaccharide structure and not of the binding specificity of the enzyme active-site. Isoamylase action on amylopectin is endo (ref. 9; T. N. Palmer, unpublished results), and the enzyme hydrolyses all (1→6)-α linkages in glycogen beta-dextrin except those linking maltosyl A chains[6,13]. Presumably, the density of branching at the periphery of the glycogen macromolecule sterically precludes the access of isoamylase to interior branch-points. Decreased density of branching (as in amylopectin) or shortened A chains (as in glycogen beta-dextrin) presumably allows access of isoamylase to interior branch-points. Two important questions remain. Does glycogen contain sparsely branched regions that are open to endo attack? Does glycogen contain 6-α-maltosyl and 6-α-maltotriosyl A chains that may be by-passed by isoamylase endo-action? With the glycogens studied, particularly oyster glycogen, the answer to each question is no. Irrespective of the degree of isoamylolysis, no measurable quantities of branched oligosaccharides were detected. It is recognised, however, that certain glycogens may contain structures compatible with endo action.

Molar distribution profiles of partial isoamylolysates. — As the hydrolysis by isoamylase of the (1→6)-α linkages in oyster glycogen proceeds as a function of time, the molecular weight distribution of released maltodextrins alters radically. The maltodextrin products of partial isoamylolysis were analysed by gel filtration on Bio-Gel P-10 and the data replotted as μmol of maltodextrin/mL *versus* $\overline{\text{d.p.}}$ (Fig. 3). At 34% debranching, the maltodextrin products of isoamylolysis had a $\overline{\text{d.p.}}$ of 6.7 and no maltodextrins of $\overline{\text{d.p.}}$ >16.5 were detected. At 41% debranching, the maltodextrin products had a $\overline{\text{d.p.}}$ of 9.6 and maltodextrins of $\overline{\text{d.p.}}$ 29 were present, whereas,

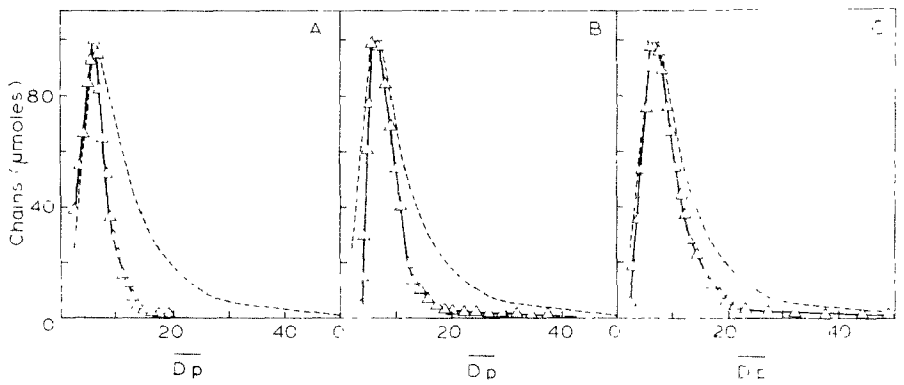

Fig. 3. Numerical distribution profiles (see Experimental) of the maltodextrin products of partial isoamylolysis of oyster glycogen: profiles obtained at 34% (A), 41% (B), and 61% (C) isoamylolysis. The dashed line shows the distribution profile at 100% debranching.

at 61% debranching, the released maltodextrins ($\overline{d.p.}$ 9.8) included material of $\overline{d.p.}$ 49. The inference is that the $\overline{d.p.}$ of released maltodextrins increases as a function of the degree of isoamylolysis. Given that isoamylase action on oyster glycogen is apparently exo, this increase in $\overline{d.p.}$ as a function of isoamylolysis implies that the length of component chains may increase dependent on their depth of attachment to the macromolecule.

Distribution profiles of the unit chains in the residual polysaccharides. — From the above discussion, it is clearly implied that the chain length ($\overline{c.l.}$) of residual polysaccharide (core polysaccharide) must be presumed to increase progressively as isoamylase sequentially cleaves component (1→4)-α-D-glucosyl chains from the periphery of the molecule. The identification of sparsely branched, residual polysaccharide in partial isoamylolysates of glycogen allowed corroboration of this point. A partial isoamylolysate of oyster glycogen corresponding to 97% debranching was chromatographed on Sephadex G-50 (see Experimental), resulting in two polyglucose peaks. These correspond, respectively, to residual polysaccharide and the maltodextrin products of enzyme exo-action. Analysis of the latter peak (for polyglucose and reducing equivalents) provides a profile of the maltodextrins released at 97% isoamylolysis. Column fractions containing residual polysaccharide were combined, and treated exhaustively with isoamylase. The resulting maltodextrins were chromatographed on Sephadex G-50. Analysis of these column fractions (for polyglucose and reducing equivalents) provided a distribution profile of the unit chains present in the residual polysaccharide at 97% isoamylolysis (Fig. 4A).

The elution profile on Sephadex G-50 of the debranched residual polysaccharide (Fig. 4A) showed that its component chains were substantially longer than the maltodextrins released by 97% isoamylolysis. The debranched polysaccharide was eluted as an approximately symmetrical polyglucose-peak (elution vol., 360 mL), whereas the released maltodextrins were eluted as an asymmetrical peak (elution vol., 410 mL)

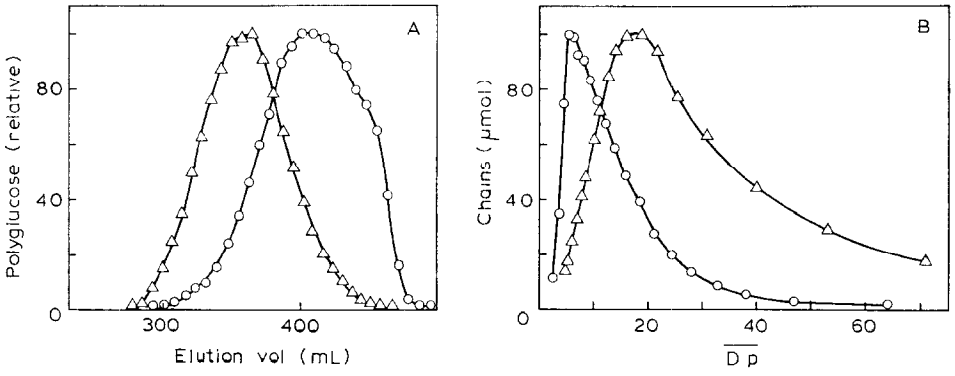

Fig. 4. The maltodextrin and residual polysaccharide products of 97% isoamylolysis of oyster glycogen. The data are shown as (A) the elution profiles and (B) the numerical unit-chain distribution-profiles of the maltodextrin products (—○—) and of the component chains of debranched residual polysaccharide (—△—).

having a distinct shoulder at 440–460 mL). Replots of the gel-filtration data as μmol of maltodextrin *versus* $\overline{d.p.}$ allows graphical analysis of chain distribution on a numerical, as opposed to a weight, basis. This type of plot (referred to as a numerical or molar unit-chain distribution-profile, see Experimental) showed that the preponderant chain species in the residual polysaccharide ($\overline{c.l.}$ 21.5) at 97% isoamylolysis (Fig. 4B) had a $\overline{d.p.}$ of 18. The profile shows right-skewed asymmetry with component chains of $\overline{d.p.} \geq 71$ present. The residual polysaccharide, although resembling amylopectin in chain length, differed from the latter in having a monodispersed chain population[1-14]. The molar unit-chain profile of the maltodextrin products (overall $\overline{d.p.}$ 10.4) of 97% isoamylolysis shows right-skewed asymmetry with the preponderant chains having a d.p. of ~6 (Fig. 4B).

At 78% isoamylolysis, the residual polysaccharide in oyster glycogen ($\overline{c.l.}$ 13.8)

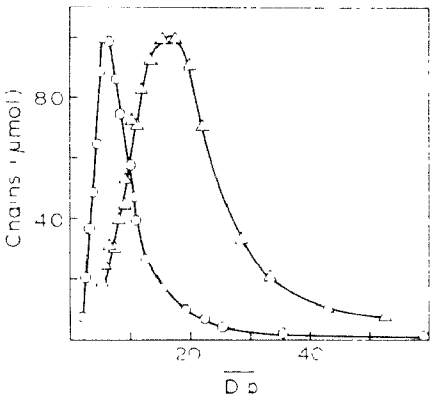

Fig. 5. Numerical unit-chain distribution-profiles of the polysaccharide ( ) and maltodextrin ( ) products of 78% isoamylolysis of oyster glycogen.

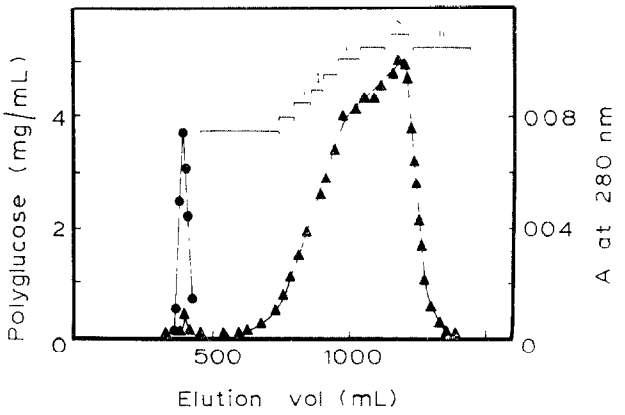

Fig. 6. Co-chromatography of isoamylase and oyster glycogen on a column (5 74 cm) of Bio-Gel P-10 at 37° and pH 5.5. The column was eluted with buffer at 0.6 mL/min, and fractions (9 mL) were assayed for polyglucose (– ▲ –), and protein (A at 280 nm, – ● –). Column fractions were combined, as designated, to give nine samples

is more densely branched than that following 97% isoamylolysis (Fig. 5). The implication is that the residual polysaccharide becomes more sparsely branched as isoamylolysis proceeds. The numerical unit-chain profile of debranched residual polysaccharide showed right-skewed asymmetry (peak $\overline{d.p.}$ 16.5), as did the corresponding profile of the maltodextrin products of 78% isoamylolysis (peak $\overline{d.p.}$ ~6).

Co-chromatography of isoamylase and glycogen. — An exo-mode of action involves an ordered and sequential hydrolysis of (1→6)-α linkages proceeding inwards from the non-reducing chain-ends. To provide a complete profile of the resulting, time-dependent release of maltodextrin products, a relatively novel approach was undertaken. To a Bio-Gel P-10 column equilibrated at pH 5.5 and maintained at 37° was applied a mixture of glycogen and isoamylase, independently pre-equilibrated at pH 5.5 and 37°, and mixed immediately prior to application to the column. The resulting elution profile is shown in Fig. 6.

Isoamylase and glycogen were eluted in the void volume of Bio-Gel P-10 (exclusion limit >20,000). In theory, maltodextrin products having M_r <20,000 will penetrate the Bio-Gel P-10 matrix and be separated from isoamylase and polysaccharide. This type of resolution should apply equally to all maltodextrin products: M_r 20,000 is equivalent to a maltodextrin of $\overline{d.p.}$ 123. The resolution of products from isoamylase/polysaccharide will continue on a time-dependent basis as column elution proceeds. Therefore, the time at which a specific maltodextrin is cleaved from the polysaccharide will determine, at least initially, its position in the elution profile. Products released last will be eluted with isoamylase and residual polysaccharide in the void volume. Fig. 6 shows a void-volume polyglucose peak consistent with incomplete isoamylolysis at the time at which isoamylase was eluted from the column. The higher the elution volume of a specific maltodextrin, the earlier, in theory, its

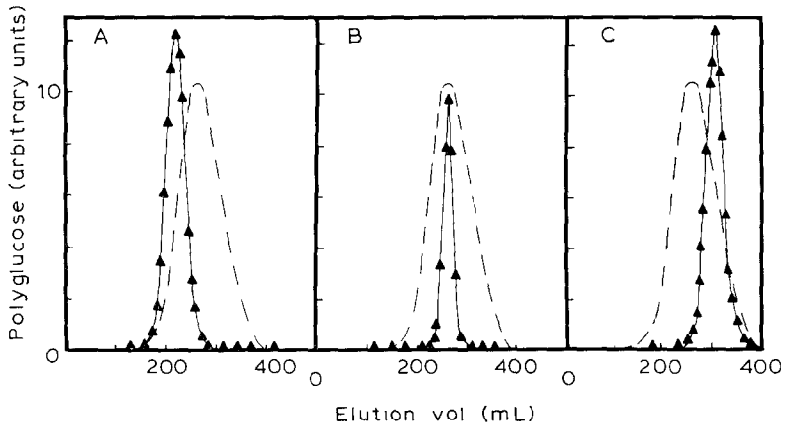

Fig. 7. Chromatography on a column (2.6 × 91 cm) of Sephadex G-50 of samples 1–9 in Fig. 6. Column fractions were neutralised (M HCl) and polyglucose was determined. Elution profiles A–C correspond, respectively, to samples 1 ($\overline{d.p.}$ 47.3), 3 ($\overline{d.p.}$ 20.1), and 8 ($\overline{d.p.}$ 6.8). The dashed line is the elution profile of completely debranched oyster-glycogen.

time of release by hydrolysis of (1→6)-α linkages; *i.e.*, elution volume is an index of time. However, gel filtration will inevitably complicate the elution profile by an M_r-dependent resolution of products. The final elution profile is therefore both time- and M_r-dependent.

Treatment (60 h, 37) of portions of column fractions with isoamylase (final conc., 0.25 U/mL) and/or pullulanase (final conc., 2 U/mL) produced no increase in reducing equivalents. This finding supports the view that isoamylase is exo-acting with glycogen as substrate. The column fractions that comprised the elution profile were combined as designated (Fig. 6), to yield nine samples for analysis. The $\overline{d.p.}$ of these samples decreased as their respective elution volumes increased. The $\overline{d.p.}$ values were 47.3 (1), 29.2 (2), 20.1 (3), 20.3 (4), 17.0 (5), 14.5 (6), 12.7 (7), 6.8 (8), and 4.9 (9). The nine samples were separately chromatographed on Sephadex G-50. Three typical elution-profiles are shown in Fig. 7. All nine profiles showed that the component maltodextrins of each sample had symmetrical weight-distribution profiles.

If fractionation on Bio-Gel P-10 were to have been exclusively time-dependent, the results would simply imply that the maltodextrin products of enzyme exo-action on glycogen increase progressively in length as a function of the degree of debranching, *i.e.*, the $\overline{d.p.}$ of component chains increases as a direct function of their depth of (1→6)-α linkage to the macromolecule. However, gel filtration inevitably distorts the fractionation of reaction products. Moreover, gel filtration of maltodextrins results in elution in order of decreasing M_r ($\overline{d.p.}$). This would contribute to (but not totally account for) precisely the type of elution profile seen in Fig. 6. Therefore, in order to verify the inference that isoamylolysis releases maltodextrins in order of increasing $\overline{d.p.}$, the effects of M_r-dependent (as opposed to time-dependent) product fractionation must be minimised. To this end, glycogen (100 mg) was chromatographed (at pH 5.5 and 37) with 9.8, 6.9, 4.8, 2.4, or 0.5 U of isoamylase (initial reaction-volume, 5 mL). Chromatography was performed on Bio-Gel P-60, which has a higher fractionation range than P-10 (3,000–60,000 as opposed to 1,500–2,000) and consequently produces less resolution of reaction products on the basis of M_r. Irrespective of isoamylase concentration, the $\overline{d.p.}$ of maltodextrins present in column fractions tended to increase as an inverse function of elution volume (results not shown).

Glycogen structure. — In our interpretation of the results presented, two basic assumptions are made. Firstly, it is assumed that isoamylase is exo-acting with oyster glycogen as substrate. Irrespective of the extent of debranching, linear maltodextrins were the exclusive products of isoamylase action on glycogens (oyster, rabbit liver, and bovine liver); branched oligosaccharide products were not detected. The implication is that glycogen itself and the polysaccharide products of its partial isoamylolysis possess structures that impose an exo-mode of action on isoamylase. As mentioned previously, it is important to recognise that the action pattern of isoamylase is determined by the structure of the polysaccharide substrate: the enzyme hydrolyses interior (1→6)-α linkages in amylopectin[9] and glycogen beta-dextrin[6,13]. The second assumption is that the primary structure of glycogen [*i.e.*, the arrangement of (1→4)-α

and (1→6)-α linkages] remains intact during the isolation of the macromolecule. It is recognised that glycogen *in vivo* may have a complex secondary/tertiary structure maintained by hydrogen bonding, protein–carbohydrate interactions, *etc.*[15]. Normal isolation procedures may disrupt this structure, but it is reasonable to assume that the primary structure remains unaltered.

The data presented show that the $\overline{\text{d.p.}}$ of maltodextrin products tends to increase as a direct function of the extent of isoamylolysis. Given our basic assumptions, it is concluded that the length of component chains in glycogen increases as a function of their depth of (1→6)-α linkage to the macromolecule. This type of chain arrangement is incompatible with the Haworth "stubbed" model for glycogen[4,16] (Fig. 8). The concept of "buried" chains[17,18] involves certain (1→4)-α-D-glucosyl chains occupying the interior spaces of the glycogen macromolecule. In theory, such chains would be relatively resistant to the action of exo-enzymes and, of importance in the current context, be shorter than chains having a similar depth of (1→6)-α attachment

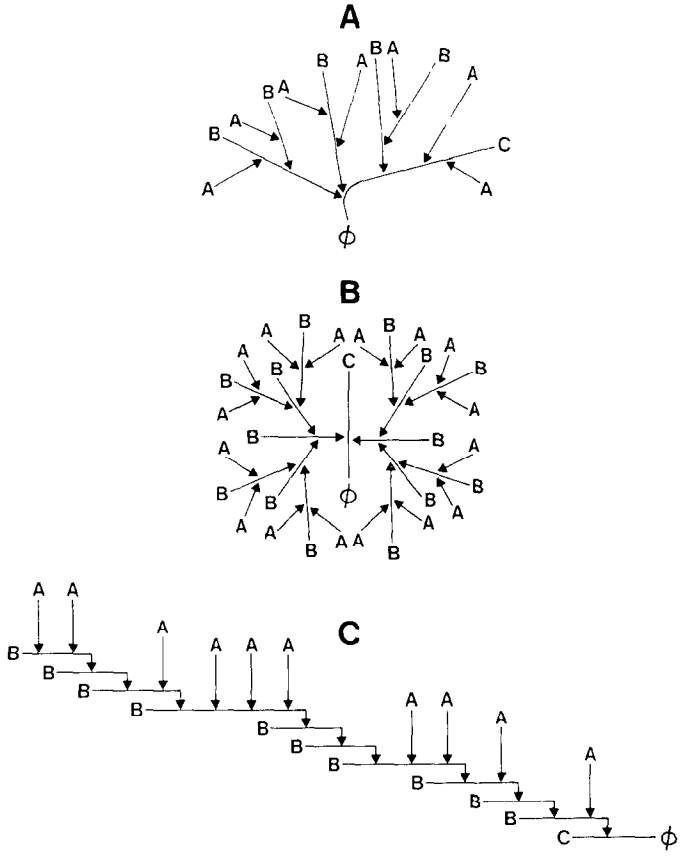

Fig. 8. Diagrammatic representations of various structures proposed for glycogen and amylopectin by Meyer and Bernfeld[2] (A), Gunja-Smith *et al.*[3] (B), and Whelan and co-workers[4,16] (C). The reducing end-groups are drawn as unsubstituted (ϕ); the (1→6)-α linkages are denoted as ↓.

to the polysaccharide but which tend to terminate at its periphery. Therefore, if "buried" chains were to exist in significant numbers, discontinuities might be expected in the distribution profiles of the maltodextrin and polysaccharide products of partial isoamylolysis. In fact, the profiles consistently showed no polydispersity. Thus, short "buried" chains must be presumed not to be present in significant numbers. The data do not preclude the existence of "buried" chains *per se*: the distribution profiles of partial isoamylolysates could easily accommodate a number of "buried" chains without noticeable distortion.

In conclusion, our results suggest that glycogen has a spherical Meyer-type structure[2,18] and that component chains tend to terminate at the periphery of the macromolecule. The data do not distinguish between the Meyer model[2] and the revised model of Gunja-Smith *et al.*[3] (Fig. 8). The latter model was advanced in view of the successive actions of isoamylase and beta-amylase on glycogen phb,beta-dextrin (*i.e.*, A chains shortened to maltosyl units by the successive actions of phosphorylase and beta-amylase). The dextrin in question contained some 6-α-maltotriosyl A-chains and therefore the underlying assumption that isoamylase action was confined to B–B branch-linkages is of doubtful validity[3].

Two points warrant comment. The first concerns the randomness of isoamylase action. In theory, hydrolase action on such a polysaccharide substrate as glycogen may be random or non-random, or a combination thereof. The mode of action is further complicated by the fact that the vast surface-area of the macromolecule (containing 10^4–10^5 non-reducing end-groups) presumably permits simultaneous attack by several enzyme molecules. It is therefore not sufficient to know simply that isoamylase action is exo. In this paper, a relatively random exo-action is assumed and certainly our conclusions would be invalid if attack were to be exclusively non-random at spatially isolated sites on the polysaccharide surface. This situation is unlikely, but verification is required. The second point is that the (1→6)-α linkages in oyster glycogen have a heterogeneous distribution. Alpha-amylase-resistant regions of dense branching ("macrodextrin") are reported to be distributed throughout the macromolecule[19]. Further studies are necessary to assess the effects of this non-random distribution of branch points on isoamylase action.

EXPERIMENTAL

Materials. — Oyster (type II) and bovine-liver glycogens were supplied by the Sigma Chemical Co. Rabbit-liver glycogen, pullulanase, and beta-amylase were products of the Boehringer Corp. Ltd. Isoamylase (a gift from Glaxo Ltd.) had a specific activity of 3.9 U/mg of protein[3,6]. Enzyme activities are expressed as μmol^{-1} min at 30°. This crude isoamylase was isolated from an organism (NCIB 9497) originally identified as *Cytophaga* and subsequently as *Polyangium*. Activity on pullulan was <0.05% of the corresponding activity on glycogen[11], and the enzyme was free from phosphorolytic, amylolytic, and α-D-glucosidase activities. Bio-Gel P-2 (200–400 mesh), P-10 (200–400 mesh), and P-60 (50–100 mesh) were obtained from

Bio-Rad Laboratories, and Sephadex G-50 medium was obtained from Pharmacia Fine Chemicals Ltd.

Chromatography of partial isoamylolysates of glycogens. — Glycogens (final conc., 50 mg/mL) were incubated at 37° with isoamylase (final conc., 0.25 U/mL) in 100mM sodium acetate buffer (pH 5.5) containing 0.1mM NaN_3. The total reaction-volumes were 100 mL. At intervals, samples were heat-inactivated (20 min, 100°) in capped Pyrex tubes and analysed for reducing equivalents[20], and polymeric glucose (polyglucose)[21] and $\overline{d.p.}$ (average degree of polymerisation) were calculated. At 100 h, enzymic hydrolysis was complete: treatment (60 h) with isoamylase (final conc., 0.8 U/mL) produced no increase in reducing end-groups. The degree of isoamylolysis (%) of samples was calculated accordingly.

Selected samples were chromatographed on a column (2.6 × 90 cm) of Bio-Gel P-10 equilibrated in 0.1mM NaN_3. Column fractions were analysed for reducing end-groups[20] and polyglucose[21].

Beta-amylolysis of partial isoamylolysates of oyster glycogen. — Heat-inactivated, partial isoamylolysates were incubated (60 h, 37°) with beta-amylase (final conc., 160 U/mL). The degree of beta-amylolysis was calculated. Portions were chromatographed on columns (2.6 × 90 cm) of Bio-Gel P-10 or P-2[12] equilibrated in 0.1M NaN_3, and fractions were analysed for reducing end-groups[20] and polyglucose[21]. Selected samples were analysed by paper chromatography[6].

Isolation of reaction products at 97% and 78% isoamylolysis. — Oyster glycogen (final conc., 100 mg/mL) was incubated overnight at 37° in 0.1M sodium acetate buffer (pH 5.5) with isoamylase (total reaction-volume, 5 mL). Two final concentrations of isoamylase were used, namely, 1.25 and 0.9 U/mL. The reaction mixtures were heat-inactivated (20 min, 100°); isoamylolysis was 97% (1.25 U of isoamylase/mL) and 78% (0.9 U of isoamylase/mL). The reaction mixtures were chromatographed on a column (2.6 × 90 cm) of Sephadex G-50 in 0.05M NaOH–M NaCl. NaOH was present to maintain the solubility of long-chain maltodextrins. The resulting elution-profiles contained two polyglucose peaks corresponding to the polysaccharide and maltodextrin products of isoamylolysis. The peak for maltodextrin of low molecular weight in each profile was analysed for reducing end-groups. The void-volume polysaccharide peaks were desalted with Bio-deminrolit (CO_3^{2-}) resin and freeze-dried. The freeze-dried material was incubated for 60 h at 37° with isoamylase (final conc., 2.5 U/mL) in 0.1M sodium acetate buffer (pH 5.5), and the resulting maltodextrins (solubilised in warm 0.05mM NaOH–M NaCl) were chromatographed on Sephadex G-50. Column fractions were analysed for polyglucose and reducing end-groups.

Co-chromatography of isoamylase and glycogen. — Solutions of oyster glycogen and isoamylase in 100mM sodium acetate buffer (pH 5.5) at 37° were mixed, to give final concentrations of 33 mg/mL and 1.25 U/mL, respectively (total volume, 15 mL), and applied to a column (5 × 74 cm) of Bio-Gel P-10 equilibrated in 100mM sodium acetate buffer (pH 5.5) at 37°. The column was eluted at 0.6 mL/min, and fractions (9 mL) were assayed for polyglucose[21]. Fractions were combined to give nine samples

(designated in Fig. 6). These samples (analysed for reducing end-groups[20] and polyglucose[21]; $\overline{d.p.}$ calculated) were desalted and freeze-dried. The freeze-dried samples, solubilised in warm 50mM NaOH–M NaCl under N_2, were chromatographed on Sephadex G-50 (column, 2.6 × 90 cm). Fractions were neutralised (M HCl), and reducing end-groups and polyglucose were determined.

Molar unit-chain distribution-profiles. – To allow graphical analysis of chain distributions on a numerical (molar), as opposed to a weight, basis, elution profiles on Bio-Gel P-10 or Sephadex G-50 were replotted. Based on polyglucose and reducing end-group analysis, the $\overline{d.p.}$ and molecular weight (M_r) of maltodextrins present in column fractions were calculated. For each fraction, the mM concentration of maltodextrins present was calculated on the basis of the ratio total polyglucose (μg/mL): M_r (allowing for the water of hydration on hydrolysis). To allow direct comparison of plots, data were normalised, *i.e.*, the preponderant maltodextrin in each plot was nominally assigned a concentration of 100 and all other concentrations were expressed relative thereto.

REFERENCES

1 J. J. Marshall, *Adv. Carbohydr. Chem. Biochem.*, 30 (1974) 257–370.
2 K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, 23 (1940) 875–885.
3 Z. Gunja-Smith, J. J. Marshall, C. Mercier, E. F. Smith, and W. J. Whelan, *FEBS Lett.*, 12 (1970) 101–104.
4 G. W. J. Matcham, N. B. Patil, E. E. Smith, and W. J. Whelan, in V. Esmann (Ed.), *Regulatory Mechanisms of Carbohydrate Metabolism, FEBS Proc. Meet., 11th*, Pergamon Press, Oxford, Vol. 42, 1977, pp. 305–315.
5 K. Yokobayashi, A. Misaki, and T. Harada, *Biochim. Biophys. Acta*, 212 (1970) 458–469.
6 Z. Gunja-Smith, J. J. Marshall, E. E. Smith, and W. J. Whelan, *FEBS Lett.*, 12 (1970) 96–100.
7 H. Akai, K. Yokobayashi, A. Misaki, and T. Harada, *Biochim. Biophys. Acta*, 237 (1971) 422–429.
8 N. A. Butler, E. Y. C. Lee, and W. J. Whelan, *Carbohydr. Res.*, 55 (1977) 73–82.
9 T. Harada, A. Misaki, H. Akai, K. Yokobayashi, and K. Sugimoto, *Biochim. Biophys. Acta*, 268 (1972) 497–505.
10 K. Kainuma, S. Kobayashi, and T. Harada, *Carbohydr. Res.*, 61 (1978) 345–357.
11 R. M. Evans, D. J. Manners, and J. R. Stark, *Carbohydr. Res.*, 76 (1979) 203–213.
12 M. John, G. Trenel, and H. Deliweg, *J. Chromatogr.*, 42 (1969) 476–481.
13 J. J. Marshall and W. J. Whelan, *Carbohydr. Res.*, 161 (1974) 234–238.
14 H. Akai, K. Yokobayashi, A. Misaki, and T. Harada, *Biochim. Biophys. Acta*, 252 (1971) 427–431.
15 W. J. Whelan, *Trends Biochem. Sci.*, 1 (1976) 13–15.
16 D. Borovsky, E. E. Smith, W. J. Whelan, D. French, and S. Kikumoto, *Arch. Biochem. Biophys.*, 198 (1979) 627–631.
17 D. French, *Baker's Dig.*, 31 (1957) 24–36.
18 D. French, in W. J. Whelan and M. P. Cameron (Eds.), *Control of Glycogen Metabolism*, Churchill, London, 1964, pp. 7–28.
19 G. L. Brammer, M. A. Rougvie, and D. French, *Carbohydr. Res.*, 24 (1972) 343–354.
20 N. Nelson, *J. Biol. Chem.*, 153 (1944) 375–380.
21 L. E. Macaskie, Ph.D. Thesis, University of London, 1977.