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Note

The unit-chain distribution profiles of branched (1→4)-α-D-glucans

T. NORMAN PALMER, LYNNE E. MACASKIE*, AND KANWALJIT K. GREWTI

Department of Biochemistry, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF (Gt. Britain)

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Isoamylase (amylopectin 6-glucanohydrolase, EC 3.2.1.68) quantitatively hydrolyses the (1→6)-α linkages in branched (1→4)-α-D-glucans[1,2]. Gel filtration of the maltodextrin products of isoamylolysis therefore defines the size distribution of component 1,4-α-D-glucosyl chains in these polysaccharides. Elution profiles show glycogen to have a symmetrical distribution of component chains, whereas the component chains of amylopectin and phytoglycogen show polydispersity[3–7]. The interpretation of these elution profiles is problematical, in that they contain an inherent weight-bias. Structural models of polysaccharides are concerned with the intramolecular arrangement of a finite number of component chains of defined lengths[3]. It is necessary, therefore, that the elution data be analysed on a numerical, as opposed to a weight, basis.

Oyster glycogen (c.l. 11.9) was debranched with isoamylase and chromatographed on Bio-Gel P-10. Column fractions were analysed for polymeric glucose and reducing end-groups, and $\overline{d.p}$ (average degree of polymerisation) and $\overline{M}_r$ (average molecular weight) were calculated. These data are plotted on a weight-basis (polysaccharide mg/mL; Fig. 1A) and on a numerical-basis [μmol of (1→4)-α-D-glucosyl chains/mL; Fig. 1B] *versus* $\overline{d.p}$. The weight-based profile shows marked asymmetry. The numerical unit-chain distribution-profile is asymmetrical, with a preponderance of long-chain material (right-skewed asymmetry). The profile has a distinct peak at $\overline{d.p}$. 8 (*cf.* c.l. 11.9); between $\overline{d.p}$. 8–45, it has almost the shape of an exponential curve. The profile is not strictly exponential; there is a fractional excess of chains of $\overline{d.p}$. 15–25. Nonetheless, the clear inference is that the numbers of component (1→4)-α-D-glucosyl chains decrease as an exponential function of $\overline{d.p}$. The numerical and weight-based profiles of rabbit-liver glycogen (Fig. 1C; c.l. 14) corroborate the conclusions reached in relation to oyster glycogen. Again, there is almost an exponential relationship between the number of component chains and $\overline{d.p}$., at least over the

*Present address, Physical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QZ, Great Britain

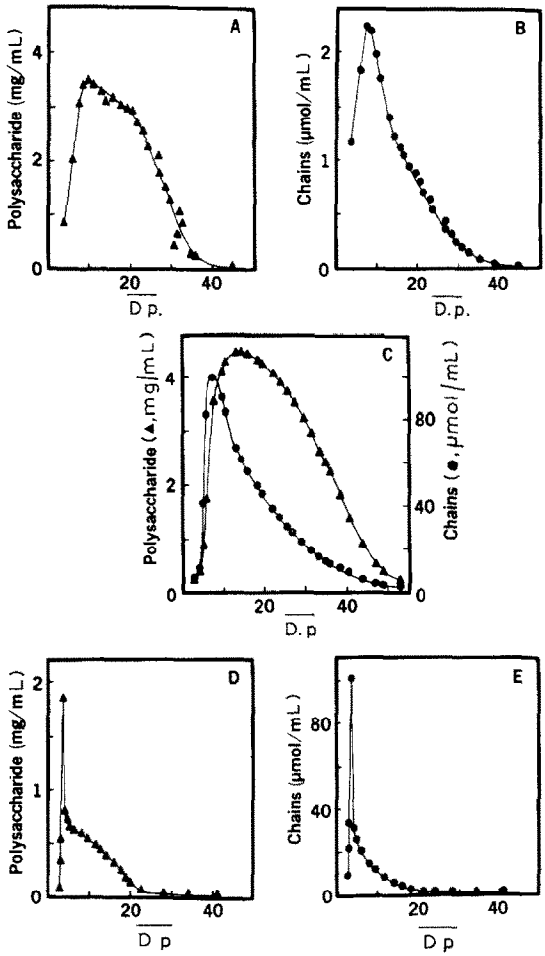

Fig. 1. Unit-chain distribution profiles of (A) oyster glycogen, μg/mL; (B) oyster glycogen, μmol/mL; (C) rabbit-liver glycogen, μg/mL (▲) and μmol/mL (●); (D) oyster-glycogen φ-dextrin, μg/mL; and (E) oyster-glycogen φ-dextrin, μmol/mL. In C and D, plots are normalised, the peak fraction being arbitrarily assigned a value of 100.

range $\overline{\text{d.p.}}$ 8–53. The data do not comply strictly to an exponential curve, as there is a fractional excess of chains of $\overline{\text{d.p.}}$ 14–29.

In glycogen phosphorylase-limit dextrin (φ-dextrin), the A and the outer portions of B chains (*i.e.*, distal to the branch points) comprise four D-glucosyl units[8]. The weight-based distribution profile of oyster-glycogen φ-dextrin (Fig. 1D) is asymmetrical to the extent that polydispersity might be inferred. By contrast, the numerical profile (Fig. 1E) shows right-skewed asymmetry with a single peak at $\overline{\text{d.p.}}$ 4 (*cf.* $\overline{\text{c.l.}}$ 6). Phosphorolysis of oyster glycogen (*cf.* Figs. 1B and 1E) results in a statistically symmetrical decrease in the $\overline{\text{d.p.}}$ of component chains in the macromolecule, *i.e.*, the distribution profile is shifted to the left. This suggests that the

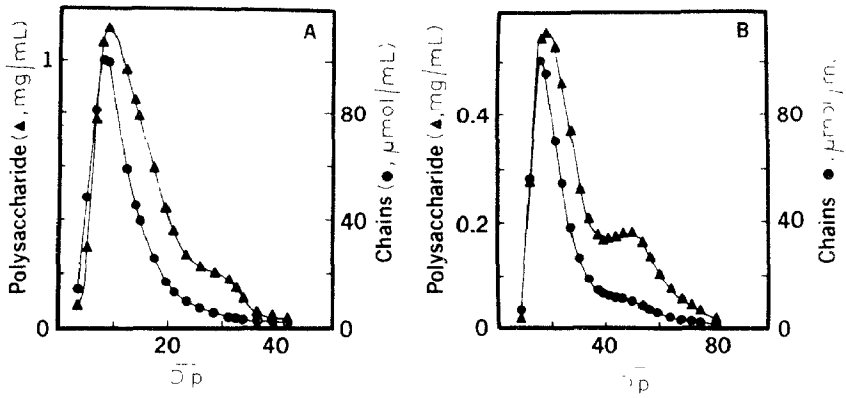

Fig. 2. Unit-chain distribution profiles of (A) sweet-corn phytoglycogen, μg/mL and μmol/mL; and (B) waxy-maize amylopectin, μg/mL and μmol/mL. The numerical profiles (μmol/mL *versus* d.p.) are normalised (see legend to Fig. 1).

majority of component chains are susceptible to phosphorylase action (*i.e.*, are not "buried"[9]). The number of chains is exponentially and reciprocally related to $\overline{\text{d.p.}}$, at least in the range $\overline{\text{d.p.}}$ 4–21. This exponential relationship was found with all glycogen-type molecules and is consistent with a spherical[9], as opposed to a linear[10], molecular structure

The weight-based distribution profile for sweet-corn phytoglycogen (Fig. 2A) shows a main peak at $\overline{\text{d.p.}}$ 9 with a shoulder at $\overline{\text{d.p.}}$ 25–35. This type of polydispersity is cited[6] as implying that phytoglycogen and glycogen differ fundamentally in molecular structure. It is now apparent that this polydispersity is artifactual, resulting simply from data analysis on a weight-biased basis. The numerical profile (Fig. 2A) shows right-skewed asymmetry with a single peak at $\overline{\text{d.p.}}$ 8. Above $\overline{\text{d.p.}}$ 8, the numbers of chains decrease exponentially with an increase in $\overline{\text{d.p.}}$, without any indication of a secondary peak. In terms of chain distribution, therefore, phytoglycogen resembles glycogen. Re-analysis, on a numerical basis, of published elution data relating to the phytoglycogens of *Anacystis nidulans*[7] and *Cecropia peltata*[11] supports this conclusion (re-plots not shown).

Amylopectins contain two distinct chain-populations by weight[3,6,12,13]. The weight-based profile of waxy-maize amylopectin (c.f. 23, Fig. 2B), for example, shows a peak at $\overline{\text{d.p.}}$ 19 and a distinct secondary peak at $\overline{\text{d.p.}}$ 50. The secondary peak accounts for ~37% of the total polysaccharide. Replotting these data on a numerical basis (Fig. 2B) does not obliterate the peak of high molecular weight. It remains as a shoulder ($\overline{\text{d.p.}}$ ~45) to the main peak ($\overline{\text{d.p.}}$ 17). Re-analysis, on a numerical basis, of published elution profiles for a variety of debranched amylopectins[3,5,12,13] confirmed a discontinuous distribution of component chains into two distinct chain-populations. The peak of high molecular weight consistently accounted for <30% of total chains. One theoretical possibility can be discounted, namely that the peak of high molecular weight comprised exclusively B chains and that of low molecular weight exclusively

A chains. In this event, the ratio of A:B chains for amylopectins would be >2, a figure not supported by published data[12,14,15], except those of Marshall and Whelan[16]. In our hands, the ratio of A:B chains for waxy-maize amylopectin was 1.42, *i.e.*, B chains constitute 41% of the total chains. The peak of high molecular weight was predominantly, if not exclusively, composed of B and C chains. This is inferred from the fact that the peak is the final product of endo-action of isoamylase on amylopectin[17,18]. These findings are entirely compatible with the "cluster model" for amylopectin[18]. This model envisages the polysaccharide to be composed of highly ordered clusters of A and B chains ($\overline{d.p.}$ 15) connected by extended B chains. The component chains of amylopectin may be arranged in a branched double-helix[10,21].

In summary, gel filtration of the products of isoamylolysis (and pullulanolysis) of branched (1→4)-α-D-glucans has proved an invaluable tool in structural analysis. However, it is clear that the correct interpretation of these elution profiles requires that they be analysed on a numerical, as opposed to a weight, basis.

EXPERIMENTAL

Materials. — The structures of the following polysaccharides were investigated: oyster glycogen (type II, Sigma), rabbit-liver glycogen (Boehringer), waxy-maize amylopectin[22], sweet-corn phytoglycogen (a gift from Dr. G. Wöber[7]), and oyster-glycogen phosphorylase-limit dextrin (ϕ-dextrin)[23]. Isoamylase (a gift from Glaxo Ltd.) had a specific activity of 3.9 U/mg of protein[2,3]. This crude isoamylase was isolated from an organism (NCIB 9497) originally identified as *Cytophaga* and subsequently as *Polyangium*. The activity on pullulan was <0.05% of the corresponding activity on glycogen[24], and the enzyme was free of phosphorolytic, amylolytic, and α-D-glucosidase activities. Enzyme units are expressed as μmol hydrolysed/min at 30°. The average chain-length[25] ($\overline{c.l.}$) of polysaccharides and the ratio of A:B chains for waxy-maize amylopectin (based on isoamylolysis of amylopectin ϕ,β-dextrin[3,12]) were determined.

Isoamylolysis of polysaccharides. — Polysaccharides were treated exhaustively (7 days, 37°) with isoamylase (final concentration, 2.5 U/mL) in 0.1M sodium acetate buffer (pH 5.5) containing 0.1mM NaN_3. Debranching was complete: addition of isoamylase (final concentration, 2.5 U/mL) and/or pullulanase (Boehringer; final concentration, 2 U/mL) produced no increase in reducing equivalents.

Chromatography of debranched polysaccharides. — Debranched polysaccharides were chromatographed on Bio-Gel P-10 (50–100 mesh, Bio-Rad Laboratories). Columns (2.6 × 90 cm) were equilibrated in 0.1mM NaN_3. Column fractions were analysed for polymeric glucose (by a combined amyloglucosidase–D-glucose oxidase reagent[26]) and for reducing end-groups (Nelson's method[27]) either directly or following concentration (by freeze-drying). Average degree of polymerisation ($\overline{d.p.}$) and average molecular weight ($\overline{M}_r$) were calculated. For each column fraction, the

maltodextrin concentration (μmol) was calculated as the ratio polyglucose (μg/mL): $\overline{M}_r$ (allowing for water of hydration on isoamylolysis). Carbohydrate recovery from columns was >95%.

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