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Occurrence of glucose polymer in undifferentiated PC12 cells

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A large glucose polymer was found, following pronase digestion, in PC12 pheochromocytoma cells metabolically labeled with [1-^{3}H]galactose. The polymer was excluded from a Bio-Gel A-0.5 m column and adsorbed by immobilized concanavalin A-Sepharose from which it was eluted with 10 mM α-methylmannoside. Glucose was found to be the sole component monosaccharide. Except for those capable of degrading glycogen, no exo- or endo-glycosidases cleaved the polymer. This is the first report on the occurrence of a glucose polymer in undifferentiated PC12 cells.

Glycogen; Pheochromocytoma; Metabolic labeling; [^{3}H]Galactose; (PC12 cell)

1. INTRODUCTION

Rat pheochromocytoma cells of the line PC12 [1] have proved to be an excellent model system for research in neurophysiology [2]. During the course of our studies on the structure of glycopeptides from PC12 cells, we found a fraction which incorporated radioactivity efficiently from [^{3}H]galactose, but only slightly from mannose and glucosamine, in the void volume of a Bio-Gel P-10 column. The material behaved like biantennary glycopeptides on concanavalin A-Sepharose, but being so large was even excluded from a Bio-Gel A-0.5 m column. The present work describes our preliminary characterization of the labeled material as a glucose polymer.

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2. MATERIALS AND METHODS

2.1. *Cells*

PC12 cells [1] were cultured in RPMI 1640 medium (Nissui Pharmaceutical Co., Japan) containing 10% heat-inactivated (30 min 56°C) horse serum (Gibco, USA) and 5% pseudo-fetal calf serum (Mitsubishi Chemical Industries, Japan) at 37°C in 5% CO_2. The cells were grown on untreated tissue culture plastic dishes (Falcon Labware, USA) and passaged once every 4 days without treatment of trypsin.

2.2. *Labeling of cells and preparation of pronase digests*

The cells were labeled for 24 h in the presence of D-[1-^{3}H]galactose (Amersham, 6.9 Ci/mmol), D-[6-^{3}H]glucosamine hydrochloride (Amersham, 40 Ci/mmol), or D-[2-^{3}H]mannose (Amersham, 11 Ci/mmol) at a concentration of 10 μCi/ml, after which they were washed, harvested in 0.85% NaCl, and boiled for 10 min. Following the addition of an equal amount of 0.2 M Tris-HCl buffer (pH 8.0, containing 20 mM $CaCl_2$) to the cells, 50 μl/ml of 1% pronase, *Streptomyces griseus* (Calbiochem, USA, autodigested at 37°C for 2 h), was added to the cell suspension and the system was incubated at 60°C under toluene. New pronase (50 μl/ml of the suspension) was added twice a day and the incubation was continued for 5 days. The digests were boiled for 10 min, and then frozen and stored at −20°C until use.

2.3. *Gel filtration and affinity chromatography*

Gel filtration was carried out using Bio-Gel P-10, P-30 or A-0.5 m columns, essentially as described in [3]. The column size was 1.0–1.2 × 90 cm and fractions of 1.1–1.2 ml were collected. Affinity chromatography on concanavalin A (Con A)-

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Sepharose 4B (Pharmacia, Sweden) was carried out as described [3] using a column of 1.0 × 15 cm at room temperature. Fractions of 1.1–1.2 ml were collected. Aliquots were subjected to radioactivity measurement as described in [4].

2.4. *Analysis for saccharide composition*

Acid hydrolysis was carried out under nitrogen with 2.5 N trifluoroacetic acid at 100°C for 5 h. Monosaccharides were then analyzed with a Gilson HPLC system using a Shodex SP1010 column (Shodex, Japan). The flow rate was 0.9 ml/min of water at 80°C. Successive fractions were collected over periods of 0.5 min. Unlabeled marker monosaccharides were run routinely in the same column and detected with a refractive index monitor.

2.5. *Digestion with glycosidases*

The digestion was carried out at 37°C for 20 h as follows: *Bacillus* sp. α-amylase (type IIA, Sigma), 1 ml of 10 units/ml in 0.1 M ammonium acetate, pH 7.0, containing 10 mM $CaCl_2$; *Rhizopus* genus mold glucoamylase (amyloglucosidase, Sigma), 250 μl of 1.5 units/ml of 0.1 M Tris-HCl, pH 7.4.

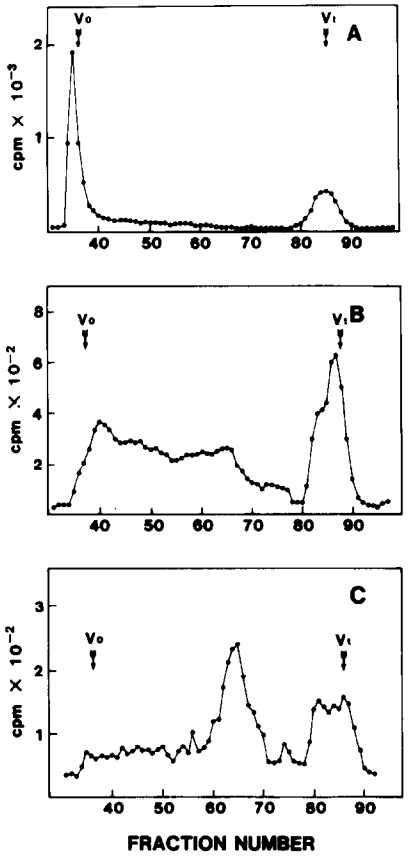

Fig.1. Fractionation on a Bio-Gel P-10 column of pronase digests of radioactive molecules from PC12 cells labeled with [^{3}H]galactose (A), [^{3}H]glucosamine (B) and [^{3}H]mannose (C).

3. RESULTS AND DISCUSSION

PC12 cells were metabolically labeled for 24 h with [1-^{3}H]galactose, [6-^{3}H]glucosamine, or [2-^{3}H]mannose and digested with pronase. The digests were fractionated on a Bio-Gel P-10 column (fig.1). Almost half (48.7%) of the total radioactivity of the galactose-labeled cells was eluted in the void volume of the column, but only 7.5% and 3.7% of the incorporation of the glucosamine and mannose labels were excluded (fig.1A–C). The degree of the incorporation of radioactivity from the different precursors suggested the presence of some molecules differing from ordinary glycopeptides. In the case of ordinary glycopeptides carrying 3 mannose, 4–6

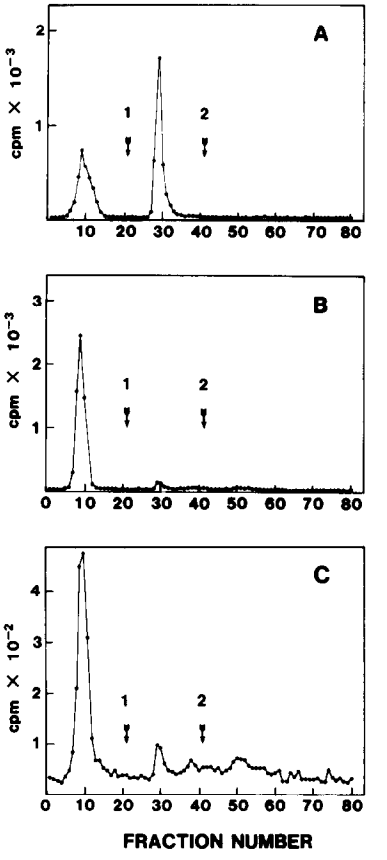

Fig.2. Fractionation on a Con A-Sepharose column of pronase digests from PC12 cells labeled with [^{3}H]galactose (A), [^{3}H]glucosamine (B) and [^{3}H]mannose (C). Arrows 1 and 2 indicate the start of elution with 10 and 300 mM α-methylmannoside, respectively.

glucosamine and 2–4 galactose residues, radioactivity should have been incorporated at essentially the same level from all precursors. But here the fraction was heavily labeled in the presence of galactose, and the radioactivity incorporated from other precursors was much less. Moreover, the radioactive material that eluted in the void volume of the Bio-Gel P-10 column was so large that it was excluded from a P-30 column (see fig.3A) and even from an A-0.5 m column (not shown).

When the pronase digests were applied to a Con A-Sepharose column, the elution profile of the galactose-labeled molecules differed markedly from the profiles of the other labeled molecules: 75.8% of the radioactivity from the galactose-labeled cells was adsorbed by Con A-Sepharose (fig.2A) and eluted from the column with 10 mM α-methylmannoside, whereas only 12.0% and 30.1% were adsorbed in the case of glucosamine- and mannose-labeled cells, respectively (fig.2B and C). 95% of the Con A-retarded molecules labeled with galactose were excluded from a Bio-Gel P-30 column (fig.3A), whereas the fraction that did not bind to concanavalin A emerged after the void volume of the Bio-Gel P-30 column (fig.3B). When chromatographed on the Bio-Gel P-30 column, Con A-retarded fractions from glucosamine- and mannose-labeled cells were eluted almost entirely after the void volume (fig.3C and D, respectively). These results clearly show the presence of galactose-labeled polymer molecules, characterized by their large size and by adsorption on Con A. The labeled material behaved like biantennary complex-type glycopeptides, which are known to be adsorbed on Con A-Sepharose and from which they are eluted with 10 mM α-methylmannoside [5]. Gel filtration (fig.3A) showed, however, that the polymer molecules were much larger than biantennary-complex-type glycopeptides.

Enzymatic digestion further indicated the unlikelihood of the large-size molecules being any glycopeptide of known structure. β-Galactosidase (jack bean), α-galactosidase (*Mortierella vinacea*)

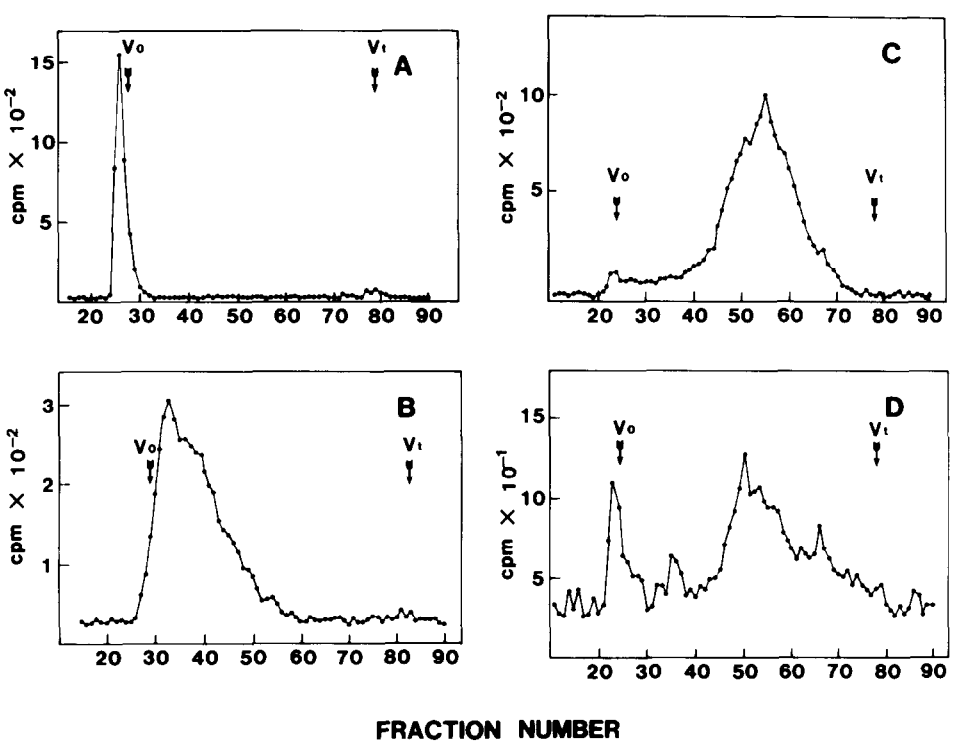

Fig.3. Fractionation on a Bio-Gel P-30 column of the Con A-Sepharose adsorbed fractions labeled with [^{3}H]galactose (A), [^{3}H]glucosamine (C) and [^{3}H]mannose (D), and of the Con A-unadsorbed fraction labeled with [^{3}H]galactose (B).

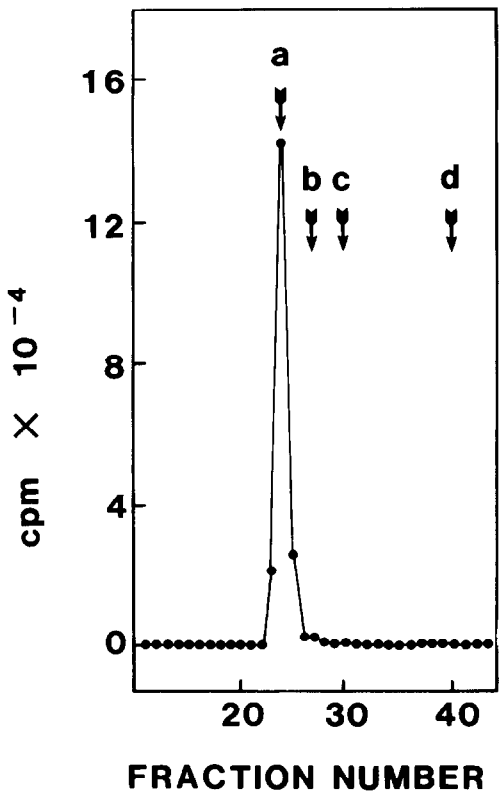

Fig.4. Analysis of the saccharide composition of the glucose polymer on a Shodex SP1010 HPLC column. Marker positions are indicated by arrows: a, glucose; b, galactose; c, mannose; d, mannitol.

and β-hexosaminidase (jack bean), separately and in all possible combinations, failed to cleave the polymer molecule (not shown). Nor was it reduced in size by almond glycopeptidase or endo-β-*N*-acetyl-glucosaminidase F (*Flavobacterium meningosepticum*).

Acid hydrolysates of the radioactive material contained glucose as the main component. HPLC with a Shodex SP1010 column (fig.4) left no ambiguity as to whether the saccharide was glucose or galactose, because the two saccharides were clearly separated on the column. Digestion of the polymer molecule with the glucose polymer-degrading enzymes, α-amylase and glucoamylase, resulted in the formation of small-size molecules and glucose, respectively, as indicated by fractionation on the Bio-Gel P-30 column (not shown). In both cases radioactivity was no longer detected at the position of the void volume of the column. The specificity of the enzymes suggested that the polymer molecules consisted of glucose units joined together by α-1,4 linkages. This is the first report on the occurrence of a glucose polymer in PC12 cells.

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