THE COMPOSITION OF GLYCOPEPTIDES, DERIVED FROM NEURAL MEMBRANES, WHICH AFFECT NEURITE GROWTH *IN VITRO*

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1. Introduction

Various proteins capable of binding to the glycoproteins of cell plasma membranes have been shown to influence cell behaviour in tissue culture [1,2]. We have previously shown that one such agent, concanavalin A, stimulated the growth of cellular processes when added to cultures of dissociated chick embryo spinal ganglia at certain concentrations [3]. This stimulation was inhibited by α-methylmannoside. It seems that concanavalin A also binds to the plasma membranes of adult neurons since it modifies the electrophoretic mobility of adult guinea pig synaptosomes [4] and agglutinates synaptosomes and synaptosomal plasma membranes prepared from adult rat brain (unpublished results).

As part of a study of possible "receptors" for concanavalin A in the neuronal plasma membrane, glycopeptides were prepared by pronase digestion of adult rat brain microsomal fractions** and subsequently fractionated by affinity chromatography and gel filtration. The aim was to test for the competitive action of these glycopeptide fractions against concanavalin A stimulation of neurite growth, but some glycopeptide fractions themselves influenced the morphology of the cultures. This paper reports these findings.

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** Such fractions should contain glycopeptides derived from the neuronal plasma membrane since approx. 20–30% of the microsomal fractions used appears to consist of fragments of the neural plasma membranes as judged by the presence of gangliosides and (Na + K)-ATPase.

2. Materials and methods

2.1. *Preparation of glycopeptides*

Microsomal fractions were collected from homogenates of adult rat brains (10 vol of 320 mM sucrose, 0.1 mM EDTA in 1 mM potassium phosphate, pH 7.6, buffer) by sedimentation between 11,500 *g* × 25 min and 25,000 *g* × 12 hr. The pellet was washed once, then defatted with chloroform : methanol [5]. The protein pellet was digested with pronase as described in detail elsewhere [6]. The glycopeptides were subsequently digested with carboxypeptidase A-DFP and leucine aminopeptidase according to Li et al. [7]. Nucleic acids and mucopolysaccharides were eliminated with cetyl pyridinium chloride as described by Brunngraber et al. [8]. The glycopeptides were then separated from most of the contaminating peptides and amino acids by gel filtration in distilled water on Sephadex G-15. The void volume peak, containing over 95% of the initial hexose, was taken as the bulk glycopeptide fraction.

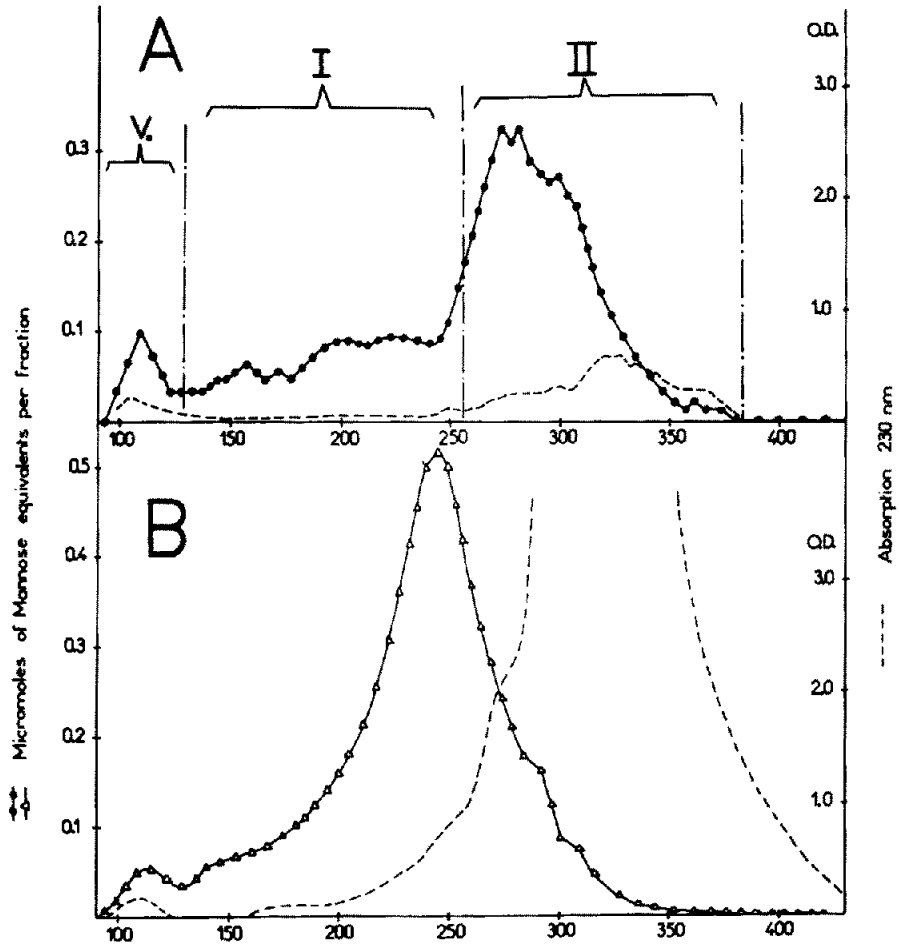

Fig. 1. Gel filtration on Sephadex G-50 (fine grade) columns (80 × 2.5 cm), equilibrated and eluted with distilled water. 5 ml samples were applied to and collected from the columns. Total hexose was determined by the phenol sulphuric acid reaction using mannose as standard. A) Concanavalin-positive glycopeptides: the eluted material was separated into two fractions as shown. The void volume material did not contain carbohydrate and was discarded. B) Concanavalin negative glycopeptides: the eluted material was pooled.

2.2. *Fractionation of glycopeptides*

Concanavalin A was prepared from Jack bean meal by the method of Agrawal and Goldstein [9] and polymerized at neutral pH with glutaraldehyde according to Avrameas and Guilbert [10]. The finely dispersed gel particles were exhaustively washed, dialysed against distilled water and suspended in $CaCl_2$, $MgCl_2$ and $MnCl_2$, each at a concentration of 1 mM.

Bulk glycopeptides dissolved in 100 ml of the same salt solution were added to the gel suspension (glycopeptides from 240 rats for 10 g concanavalin A polymerized) and agitated overnight. The suspension was centrifuged (11,000 *g* × 2 hr). The pelleted gel particles were then washed twice with 150 ml of salt solution, and the pooled supernatants were freeze-dried, then desalted on SephadexG-15 (concanavalin-negative glycopeptides). The adsorbed glycopeptides were then eluted overnight with 0.1 M α-methylmannoside from the gel particles. The elution process was repeated twice . The pooled supernatants were freeze-dried and freed of α-methylmannoside by repeated gel filtration on Sephadex G-15 (concanavalin-posi-

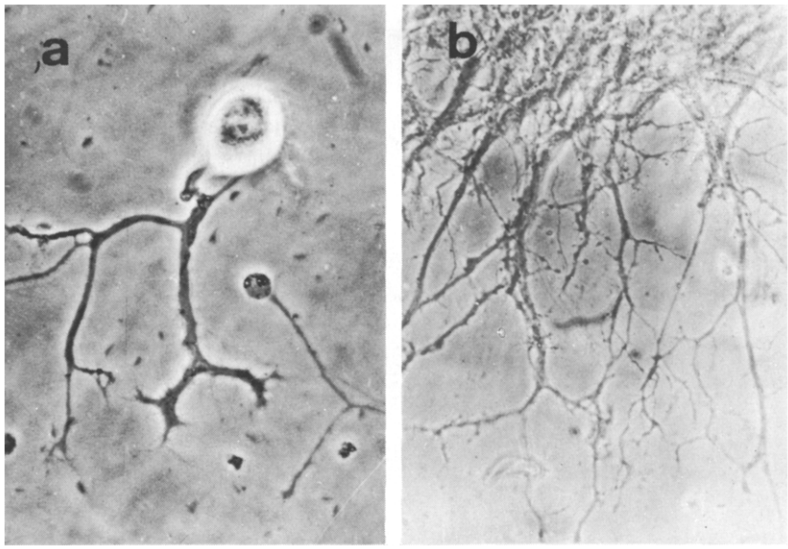

Fig. 2. Morphology by phase contrast microscopy of stimulated cultures of dissociated chick embryo spinal ganglia. a) A typical stimulated neuron: magnification 320 ×. b) Portion of the periphery of a stimulated ganglion fragment: magnification 130 ×.

tive glycopeptides). Both fractions of glycopeptides were then chromatographed on Sephadex G-50.

2.3. *Tissue culture*

Dissociated spinal ganglia from 12 day old chick embryos were cultured without glucose as described previously [3]. Fractions to be tested for biological activity were dissoved in Tyrode solution and sterilized by Millipore (0.45 μm) filtration.

2.4. *Analytical methods*

Total hexose was determined by the phenol–sulphuric acid reaction [11], using mannose as standard. Individual carbohydrates were determined by gas liquid chromatography of the trifluoroacetate derivatives of the O-methyl glycosides formed by methanolysis of the glycopeptides [12].

3. Results and discussion

The concanavalin-positive glycopeptides were fractionated on Sephadex G-50 into a long, high molecular weight trail followed by a low molecular weight glycopeptide peak (fig.1A). The material was divided into two fractions as shown. Concanavalin-negative glycopeptides were eluted from Sephadex G-50 as a broad peak (fig.1B). The carbohydrate ratios of the bulk microsomal glycopeptides and the various sub-fractions are given in table 1. The concanavalin-negative glycopeptides contained mannose and *N*-acetylglucosamine, most of the sialic acid and fucose and all of the galactose and *N*-acetylgalactosamine of the bulk glycopeptides. By contrast the two concanavalin-positive fractions contained much less fucose and sialic acid. Indeed the concanavalin-positive II fraction was devoid of sialic acid. This fraction was of particular interest since in chromatographic properties and carbohydrate composition it corresponded to a glycopeptide fraction isolated from the synaptosomal plasma membrane [13,14]. Both concanavalin-positive fractions were enriched in mannose relative to glucosamine.

When the various fractions were tested for their effects on cultures of dissociated chick embryo spinal ganglia, fraction II of the concanavalin-positive glycopeptides stimulated them in a fashion similar to concanavalin A itself. The characteristic morphology of stimulated isolated cells is shown in fig. 2A. The normally round cells have long, thick, highly branched processes which seem to have varicosities. The percent of cells with this type of

Table 1
Carbohydrate molar ratios of microsomal glycopeptide fractions.

Sugar	Glycopeptide fraction			
	Bulk	Concanavalin negative	Concanavalin positive I	Concanavalin positive II
Sialic acid	0.43	0.85	0.25	0.00
Fucose	0.43	0.62	0.22	0.22
Galactose	0.55	0.65	0.00	0.00
Mannose	1.09	0.63	2.25	3.74
N-Acetyl galactosamine	0.23	0.42	0.00	0.00
N-Acetyl glucosamine	1.00	1.00	1.00	1.00

Carbohydrate compositions were determined by gas liquid chromatography of the trifluoroacetate derivatives of the *O*-methyl glycosides formed by methanolysis of the glycopeptide fractions. Meso-inositol was used as internal standard.

morphology was doubled in the presence of concanavalin A and the fraction II concanavalin-positive glycopeptides. The concanavalin A effect was maximal at around 12 μg/ml [3] and the glycopeptide effect at around 50–100 nmole glycopeptide mannose/ml. Higher concentrations of both agents caused degeneration.

Another feature of the stimulation could be clearly seen on ganglion fragments (fig. 2B). Complex networks of nerve fibres and elongate satellite Schwann-like cells were visible at the periphery of the fragments. Migration or multiplication of this type of cell seemed to be stimulated.

Only separation and testing *in vitro* of more defined glycopeptide fractions, and comparison with the effects of glycopeptides of known structure isolated from known glycoproteins will establish if there is a strict structural specificity involved in these phenomena. We cannot explain at present why concanavalin A, and glycopeptides binding to concanavalin A have similar effects on these cultures of dissociated chick embryo spinal ganglia. However the simplest hypothesis is that, if these phenomena are related to control of growth and differentiation mediated by cell–cell contact, the presence of a glycoprotein receptor (from which the glycopeptides are derived, to which concanavalin A binds) implies a site on the other cell (which concanavalin A mimics) capable of reacting with the glycoprotein. Binding to the glycoprotein receptor (either by the natural binding site or by concanavalin A) or blocking of the binding site (either by the glycoprotein receptor or the glycopeptide) would have similar effects on cell behaviour.

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