Vol. 108, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 16, 1982 Pages 385-391

THE COMPARISON OF AN ACIDIC GLYCOPEPTIDE FROM THE ASCITIC TUMOR FLUID AND OF THE GLYCOPEPTIDE FROM EHRLICH CELLS

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Received August 2, 1982

SUMMARY. An acidic glycopeptide of molecular weight of 12000 daltons and of pI 5.6 from the ascitic tumor fluid of Ehrlich cells was isolated and characterized with regard to its physico-chemical properties and compared with an acidic glycopeptide of Ehrlich cells. Both glycopeptides have the same carbohydrate and amino acid components but differ in respect of quantity. The origin of an acidic glycopeptide from the ascitic tumor fluid of Ehrlich cells is briefly discussed.

INTRODUCTION

It is well known that polypeptides of molecular weight of 7000 - 20000 daltons are important factors regulating the growth of cells in culture / 1 /. Animal cells that are malignantly transformed usually exhibit diminished requirements for polypeptide growth factors in culture / 2-4 /. This change appears to be one of the first among many phenotypic alterations observed after transformation / 5 /.

Previously, we have isolated and purified an acidic glycopeptide from cytosol of Ehrlich cells and studied some of its physico-chemical and biological properties / 6 /. We have found, that this glycopeptide stimulates cell division in mouse BALB c/3T3 fibroblasts / 7 / and their multiplication / 8 /.

Several studies have shown that a variety of cultured cells secrete or shed glycopeptides into their growth media. Those polypeptides can also be released of viral transformed mouse cells and certain human tumor cells / 1, 9, 10 /. They

0006-291X/82/170385-07$01.00/0

can be extracted from cells in culture or from tumor growing in animals / 11 /.

The present paper describes the isolation of an acidic glycopeptide directly from the ascitic tumor fluid of Ehrlich cells and deals with the comparison of its physico-chemical properties with the one which appeared in Ehrlich cells.

MATERIALS AND METHODS

Ehrlich ascites tumor was maintained in vivo by weekly intraperitoneal transplantation approximately of 5 x 10^6 cells into Swiss white mice weighing 30 - 40 g. Cell-free ascites fluid was prepared by centrifugation of the ascitic fluid for 10 min at 4° at 1000 x g and the supernatant was collected.

Ascitic tumor fluid was at first submitted to the gel filtration on Sephadex G-25 column /3.5 x 60 cm/ in order to separate the high molecular weight from the low molecular weight substances.

The second peak, containing the low molecular weight substances was pooled, concentrated and desalted on the column with Bio-Gel P-2 /Bio-Rad Laboratories, Richmond, California/. The column was eluted with 0.01 M ammonium bicarbonate and the fraction of 3 ml were colected and assayed for absorption at 230 nm.

The fractionation of polypeptide mixture was carried out by chromatography on the cationic exchanger SP-Sephadex C-25, as described previously for the fractionation of cytoplasmic polypeptides of Ehrlich cells / 6 /.

Molecular weight determination of glycopeptide was carried out by electrophoresis on 12.5% polyacrylamide slab gels in discontinuous buffer system according to Laemmli /12/. The following polypeptides were used as standards: insulin m.w. 6000 /Reanal, Budapest, Hungary/, ribonuclease A m.w. 13700 /Pharmacia Fine Chemicals, Uppsala, Sweden/, chymotrypsinogen A m.w. 23500 /Pharmacia Fine Chemicals, Uppsala, Sweden/. The gels were stained with Coomassie Brilliant Blue R-250 according to Fairbanks / 13 /.

The isoelectric focusing of polypeptide was performed in a 110 ml column /LKB, Bromma, Sweden/ according to Westeberg and Swennson / 14/ for 30 h at 4° using 2.5% Pharmalyte gradient /pH 3.0 - 10.0/. 2 ml fractions were collected and the pH and α-amino nitrogen was determined.

Amino acid analysis was carried out after hydrolysis in 6 N HCl at 120° for 18 hours with Microtechma autoanalyser, model AAA 881, Praha, Czechoslovakia.

Hexoses were identified and quantitatively estimated by gas liquid chromatography. The instrument used was Beckman GC2 chromatograph equipped with a flame ionisation detector and Honeywell-Brown 0-1 mV recorder / 15 /.

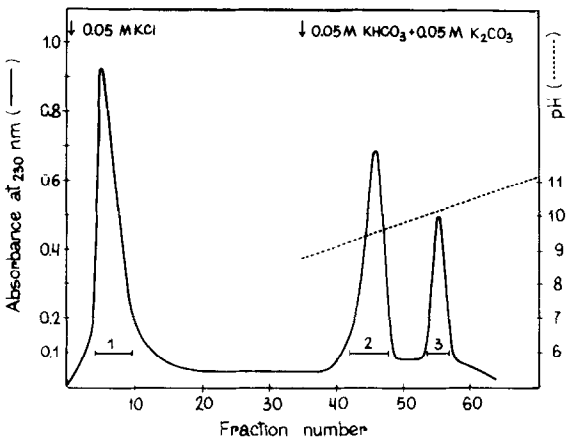

Fig. 1. SP-Sephadex C-25 chromatography of polypeptide mixture of the ascitic tumor fluid.

RESULTS AND DISCUSSION

Three peaks from the fractionation of low molecular substances of ascitic fluid were obtained /Fig. 1/. The first one was eluted at void volume and contained carbohydrate component. It had isoelectric point at pH 5.6 /Fig. 2/ and was called an acidic glycopeptide. The remaining two peaks eluating with the gradient of pH were devoid of sugar and sialic acid and were basic in character. From the elution pattern it can be seen, that an acidic glycopeptide is the most abundant polypeptide among the low molecular substances

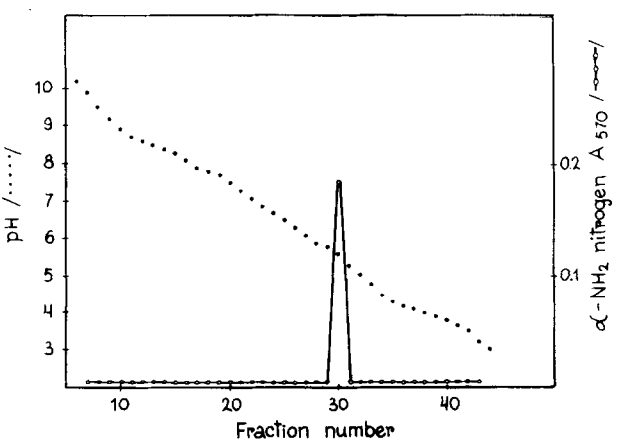

Fig. 2. Isoelectric focusing of an acidic glycopeptide from the ascitic tumor of Ehrlich cells.

Table 1: Some physico-chemical properties of acidic glycopeptide from the ascitic tumor fluid and of glycopeptide from Ehrlich cells

	pI /pH/	Molecular weight /daltons/	Carbohydrate component[x]				
			Glucose	Galactose	Mannose	Galacto-samine	Sialic acid
Glycopeptide from the ascitic tumor fluid	5.6	12 000	101.8	27.6	89.6	30.1	3.5
Glycopeptide from Ehrlich cells	5.0	18 500	150.1	69.2	161.1	80.2	33.2

[x] μg of carbohydrate component per mg of α-amino nitrogen

Table 2: Amino acid composition of an acidic glycopeptide from the ascitic tumor fluid and of glycopeptide derived from Ehrlich cells

Amino acid	An acidic glycopeptide[x] of ascitic tumor fluid	of Ehrlich cells
lysine	10.7	12.6
histidine	11.2	16.9
arginine	51.0	86.1
aspartic acid	179.1	181.2
threonine	100.2	103.0
serine	87.0	89.2
glutamic acid	110.7	113.1
proline	26.5	30.6
glycine	63.5	92.8
alanine	79.6	111.2
valine	30.2	70.1
isoleucine	21.3	30.3
leucine	37.3	63.6
tryptophan	N.D.	N.D.

[x] Residues/1000 residues

of ascitic tumor fluid. The amount of this polypeptide calculated from recovery of 20 mg sample of α-amino nitrogen is about 40%.

Some physico-chemical properties of an acidic glycopeptide isolated from the ascitic tumor fluid were presented in Table 1, in comparison with this acidic glycopeptide obtained from Ehrlich cells. An acidic glycopeptide from the ascitic tumor fluid possesses higher isoelectric point /pH - 5.6/, lower molecular weight /12000 daltons/ and diminished content of carbohydrate component /particularly of sialic acid/ and amino acid component than glycopeptide isolated from Ehrlich cells.

Results of amino acids analysis of both acidic glycopeptides are presented in Table 2. The polypeptides contain

considerable and similar amounts of aspartic and glutamic acids, threonine and serine. The amount of remaining amino acids including the basic amino acids are diminished.

It should be stressed that the composition of two acidic polypeptides both with regard to amino acid and carbohydrate component exhibit large similarities. The presence of the same sugar and amino acid components in both polypeptides, at reduction of their amounts, can suggest that an acidic glycopeptide of ascitic tumor fluid originate from Ehrlich cells. It seems probable that this glycopeptide is released from the tumor cells to the ascitic tumor fluid and subsequently degraded in the extracellular medium to lower molecular weight substance. This suggestion is in good agreement with the data of Lage et al. / 16 / who demonstrated high activity of proteases and glycosidases in ascitic tumor fluid of Ehrlich cells. The reduction of sialic acid in the glycopeptide derived from the ascitic tumor fluid might explain the increase of isoelectric point.

It should be emphasized that the most of the secreted polypeptides from eukaryotic cells have glycopeptide character. Carbohydrate components with sialic acid enable the polypeptide penetration across the membrane to the extracellular spaces, and facilitate the peptide recognition by suitable receptors of destination cells. Furthermore, covalent attachment of carbohydrate component to the peptide part of molecule may serve some specific biological function of glycopeptide.

It is not excluded that during the migration of glycopeptide by the endoplasmic reticulum channels and by Golgi cisternae comes to the limited degradation of molecule and to the rapid liberation from the cells. It is known that those organs of cells play the role in the production and processing of secretory proteins and polypeptides / 17 /. The further studies to resolve this problem are in our laboratory in progress.

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