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THE ARGININE-RICH PEPTIDE FROM CYTOSOL OF GUERIN EPITHELIOMA

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Summary: Arginine-rich peptide from cytosol of Guerin epitheliomas has been isolated and characterized. It contains 212.0 arginine residues, 77.1 lysine and 36.9 histidine residues per 1000 residues of amino acids. The ratio of basic to the acidic amino acids amounted to 1.23. The peptide has a cationic character with an isoelectric point of 8.6. No carbohydrate component was found in this peptide. It is suggested that this peptide originates from decomposition of arginine-rich basic protein stated previously in cytosol of this tumor.

INTRODUCTION

The existence of arginine-rich basic protein in Guerin epithelioma in rats has been reported previously /1/. This protein is *in vitro* very easily degraded by extract from Guerin tumor lysosomes /2/. It can be supposed that this protein is a proper substrate for cellular acid proteases. On the other hand, a low-molecular arginine-rich peptides in the blood of tumor bearing rats have been observed /3/.

These findings induced the authors to study *in vitro* the low molecular peptides in Guerin epithelioma cytosol looking for arginine-rich peptides, which may originate from the degradation of this protein in tumor cells.

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MATERIALS AND METHODS

Guerin epithelioma was grown on male Wistar rats for 30 days. Tumors were transplanted in 20 rats in a manner previously described /3/. Tumors were homogenized for 2 min. in the medium containing 0.25 M sucrose, 0.03 mM Tris-HCl, 0.02 mM EDTA pH 7.4 in a volume which yields 10 % of homogenate.

Cytosol fraction of Guerin epithelioma was obtained by centrifugation of post-mitochondrial supernatant at 105 000 x g for 60 min. The cytosol was deproteinized with perchloric acid /10 %/, which was then neutralized with potassium hydroxide. The insoluble sediment was discarded and remaining material was concentrated by lyophilization. The separation of peptides was performed by chromatography on the SP-Sephadex column.

α-amino nitrogen content was determined with ninhydrin reaction according to Rosen, described by Devenyi and Gergely /4/.

Carbohydrate components of peptides were determined by the antrone method /5/ using equimolar amounts of glucose and galactose as a standard.

Hexosamines were determined according to Belcher et al. /6/.

Sialic acid was measured after hydrolysis of peptide by the thiobarbituric acid procedure described by Warren /7/.

The isoelectric focusing of basic peptide was performed at 4° within 48 hours with the use of Pharmalyte, pH 5.0 - 9.0, produced by Pharmacia Fine Chemicals. 110 ml LKB column was used /8/.

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

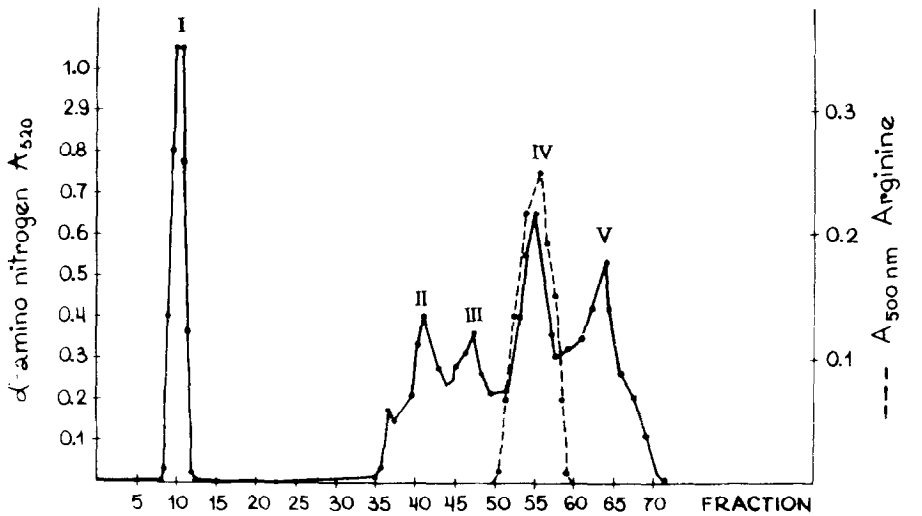

Fig. 1. SP-Sephadex chromatography of deproteinized cytosol of Guerin epithelioma. The samples were applied to the column /1.8 x 28 cm/ previously equilibrated with 0.05 M KCl and eluted with the same solution. After collecting 75 ml of the effluent the elution was continued with the mixture of 0.05 M $KHCO_3$ + K_2CO_3.

RESULTS AND DISCUSSION

Our studies demonstrate the presence of arginine-rich basic peptide in cytosol of Guerin epithelioma. Figure 1 shows the fractionation of peptide mixture on SP-Sephadex column into five peaks. Peak IV is an arginine-rich peptide and it has an isoelectric point at 8.6 Fig. 2/. Results of amino acid analysis are presented in the table. The peptide contains 212.0 arginine residues, 77.1 lysine and 36.9 histidine residues per 1000 amino acid residues. The ratio of basic to acidic amino acids is equal to 1.25 and that of arginine to lysine is equal to 2.78. No neutral sugars, hexosamines and sialic acid were found.

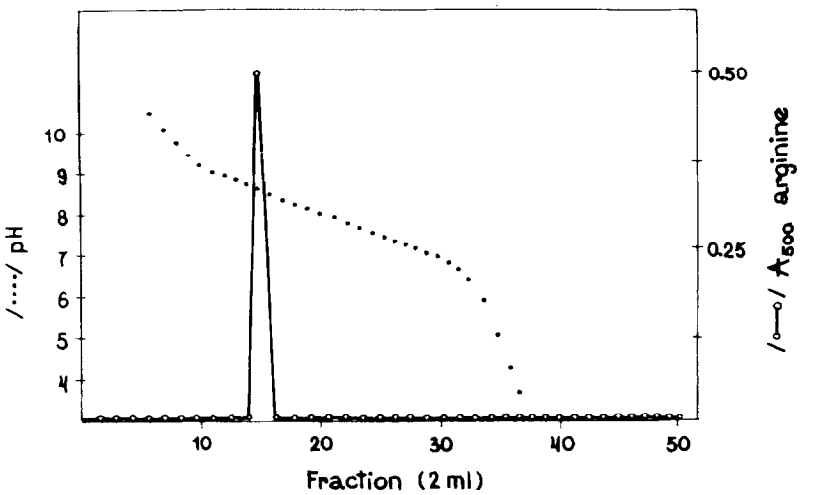

Fig. 2. Isoelectric focusing of arginine-rich peptide from the cytosol of Guerin epithelioma.

TABLE 1
Amino acid composition of arginine-rich peptide from cytosol of Guerin epithelioma

Amino acid	Residues/1000 residues
lysine	77.1
histidine	36.9
arginine	212.0
glycine	208.7
aspartic acid	91.5
glutamic acid	172.7
alanine	181.9
leucine	19.1
tryptophan	not determined

It is worthy of note that the arginine-rich basic peptide from Guerin epithelioma cytosol contains a small variety of amino acids in contrast to the amino acids composition of arginine-rich basic protein from cytosol. However, the amino acid composition of the

peptide is very similar to that of low molecular peptide which appears in the blood plasma in a form bound with albumin of tumor bearing rats /9/.

The peptide gives a positive ninhydrin reaction and penetrates through cellophane membranes in contrast to the other peptides present in the tumor cytosol. It is not precipitable by trichloroacetic, perchloric and sulfosalicylic acids and gives only a trace reaction in the procedure of Lowry et al. /10/. The absence of aromatic amino acids, and the presence of large amounts of hydrophobic amino acids /glycine, alanine and leucine/ in the peptide are of interest.

It seems possible that this peptide originates from the degradation of arginine-rich protein by intracellular proteolytic enzymes and that it enhances the permeability of tumor cell membranes. It is likely that this peptide penetrates into the extracellular spaces and from there into the blood stream where it becomes coupled with plasma albumins.

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