Cytoplasmic Nucleopeptides in Guinea Pig Liver

Nucleopeptides have been isolated from a variety of biological materials. They have been found in yeasts[1], *Streptococcus fæcalis*[2] and *Chlorella*[4]. There is also some record of the existence of fractions of ribonucleic acid containing peptides in a bound form[3].

In this communication the subfractionation of dialysable nucleopeptides from guinea pig liver is reported and certain properties of the subfractions described.

Ground liver tissue (6 gm.) was homogenized with an equal weight of 0·25 *M* sucrose solution, the dispersion diluted with more sucrose up to ten times the original weight of the tissue, and then centrifuged at 0° and 10,000 *g*. The supernatant was saturated with solid ammonium sulphate, centrifuged in the cold at 32,000 *g*, and the precipitate discarded. The supernatant was treated with cold ethanol to a final concentration of 85 per cent and the precipitated ammonium sulphate removed by centrifugation. The ethanol was then removed by distillation *in vacuo* and the concentrated material passed through a glass filter (Schott *G* 4). From this concentrated solution a sample corresponding to 1·2 mgm. of ribonucleic acid (as estimated by absorption at 260 mμ) was withdrawn, diluted with water to 10 ml. and transferred to a column (1·5 cm. in diameter and 12 cm. long) containing triethylaminoethyl cellulose[5]. The fractionation was carried out by elution at 8–10°, first with 0·01 *M tris* buffer *p*H 7·4, and then with the same buffer but containing increasing concentrations of sodium chloride. Afterwards the column was eluted with 1 *M* sodium chloride and finally with 1 per cent sodium hydroxide. The ribonucleic acid content of the eight fractions thus obtained was followed by measuring the light extinction at 260 mμ. The results are given in Fig. 1.

All eight fractions were examined for the presence of purine and pyrimidine bases, ribose, amino-acids, and activated amino-acids or peptides. For the identification of purine and pyrimidine bases samples were evaporated to dryness, hydrolysed for 1 hr. at 100° with 12 *N* perchloric acid, and after elimination of perchloric acid subjected to chromatography, using hydrochloric acid:*iso*propanol:water[6] and formic acid: butanol-water[7] as solvents. The presence of adenine, guanine, cytosine, uracil, and thymine was detected in all eight fractions. For the identification of ribose chromatography was used after the samples have been treated with bromine water[8]. Amino-acids were demonstrated by the reaction with ninhydrin[9]; this was positive in all eight fractions. On hydrolysis of the samples it became considerably stronger thus indicating the presence of peptides.

On treatment with salt-free hydroxylamine and acid ferric chloride solution a pink-brown colouration developed in all fractions, due to the formation of hydroxamic acids. The presence of carboxyl-activated amino-acids or peptides was examined further by treating the samples with hydroxylamine and subjecting them to chromatography. After treatment with ferric chloride 6–8 hydroxamate spots were revealed. The spots were excised, eluted with 0·1 hydrochloric acid and the eluates hydrolysed for 16 hr. with 6 *N* hydrochloric acid at 105°. In each spot the following amino-acids identified: cysteine, tyrosine, glycine, glutamic acid, methionine, aspartic acid, histidine, lysine, serine, alanine, valine, phenylalanine and leucine.

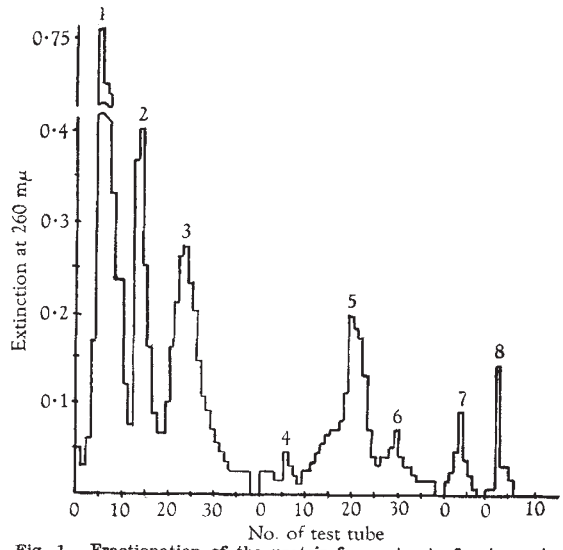

Fig. 1. Fractionation of the protein-free extract of guinea pig liver on a column filled with triethylaminoethylcellulose. Eluents used: fractions 1–3, 0·01 *M tris* buffer, *p*H 7·4; fractions 4–6 0·01 *M tris* buffer containing increasing concentrations of sodium chloride; mixing camer, 0·51. Fraction 7, 1 *M* sodium chloride solution in 0·01 *M tris* buffer; fraction 8, 1 per cent sodium hydroxide. Period of collecting each fraction: 8–10 min. Vol. of each fraction: 3·8 ml.

Paper electrophoresis carried out in each fraction on Whatman paper No. 1 in 0·1 *M* ammonium acetate buffer *p*H 5·9 at 0°, 15 hr., yielded 2–3 spots which showed violet fluorescence in ultra-violet light. Those spots which moved towards the cathode produced pink-brown colour on treatment with hydroxylamine and ferric chloride solution, thus indicating the presence of carboxyl-activated peptides. The spots moving towards the anode did not develop any colour under the same conditions. Thus it could be seen that the fractions contained some free nucleic substances as well as ones bound to peptides.

The eluates from single cathode-moving spots were treated with hydroxylamine and hydroxamates were chromatographed. After treatment with ferric chloride, 2–4 pink-brown spots were revealed. This result might indicate that one nucleotidic moiety was bound with a couple or more of peptides, providing the homogenity of each electrophoretic spot.

The above results indicate that protein-free extracts from guinea pig liver contain certain oligonucleotides, each attached to 2–4 carboxyl-activated peptides.

The possibility that such complexes may be involved in the protein biosynthesis is now under investigation.

A more detailed account of the experiments will be published in the *Acta Biochimica Polonica*.

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