Evidence for peptides in RNA prepared by phenol extraction

It was recently shown that RNA, prepared by phenol extraction from tobacco-mosaic virus[1], from ascites-tumour cells infected with Mengo encephalitis virus[2], from mouse brains infected with Eastern equine encephalitis[3] and Semliki Forest virus[4], and from poliovirus[5] are infectious. The preparations of RNA used in these experiments did not contain significant amounts of virus particles and showed an upper limit of protein contamination of 0.02 % according to serological tests[1]. Ribonuclease treatment rapidly reduced the infectivity of such preparations, whilst intact virus particles were not affected[4]. Thus, RNA appears to be able to transfer all the genetic information necessary for the reproduction of specific virus nucleic acids and proteins inside the attacked cells. Therefore, the investigation of all structural details of such native RNA preparations is of considerable biological interest. It is generally believed that nucleic acids are built up from nucleotides only, but it is possible that some other compounds are built into their macromolecular structure. It is well known from experiments on the role of RNA in amino acid incorporation into proteins that RNA prepared by phenol extraction contain amino acids[6-9]. In this communication, the presence of peptides in RNA, prepared by the same method, is reported.

RNA from baker's yeast, Ehrlich ascites-tumour cells, and mouse liver and brain, were prepared by phenol extraction according to WECKER AND SCHÄFER[3]. The preparations were several times precipitated by 70 % ethanol, containing sodium acetate. Material reacting with the biuret reagent[10] and with the phenol reagent[11] amounted to about 0.2–1.5 %. This could not be removed even after dialysis against distilled water for several days, repeated precipitation by NaCl at pH 2.0, treatment with 2 *M* hydroxylamine at pH 7.6, or a short hydrolysis by 0.2 *N* KOH. The amount of this material was decreased, but it was not completely removed by repeated treatment with 1 % sodium dodecylsulfate at pH 8.0 and 80°, which resulted in the depolymerization of RNA.

When RNA was digested by means of pancreatic ribonuclease, dialysable material of peptide character, reacting only slightly with ninhydrin, but yielding amino acids when hydrolysed with HCl, was gradually released. In the usual chromatographic systems and on paper electrophoresis the released material behaved like peptides. By means of the dinitrophenyl technique it was found that about 20 to 40 % of the amino acids present in RNA showed free amino groups. Some peptides remained bound to the non-digested core of the RNA.

In the hydrolysates of RNA, prepared with 6 *N* HCl at 105° for 12–14 h, glutamic acid, aspartic acid, arginine, lysine, leucine (isoleucine), serine, threonine and valine beside a smaller amount of glycine, α-alanine, phenylalanine, cystine, methionine and some traces of other amino acids were found on paper chromatograms. Peptides, released by the ribonuclease treatment, contained the same amino acids. It is very interesting that the acidic amino acids predominated in all the hydrolysates.

Preparations from which a large part of the peptide ingredient was removed by detergent treatment, contained glutamic acid, aspartic acid and serine beside a smaller amount of lysine, leucine and some traces of other amino acids.

Therefore it appears that the preparations of RNA, prepared by phenol extraction, contain a quite significant amount of substances of peptide character, which are intimately connected with various parts of the RNA macromolecule. The form of

chemical binding between these peptides and RNA is not yet clear. KONINGSBERGER, VAN DER GRINTEN AND OVERBEEK reported the presence of carboxyl-activated peptides in yeast microsomal ribonucleoprotein particles[12]. In our experiments there is evidently no question of RNA-bound carboxyl-activated peptides, as after hydroxylamine treatment we could find only very small amounts of hydroxamic acids.

The presence of peptides in RNA is not conditioned by only a certain method of preparation of the RNA. KEIL AND HRUBEŠOVÁ who stressed the peptide contamination in pancreatic RNA prepared by the thermal denaturation of corresponding nucleoproteins obtained similar results[13]. The presence of amino acids and compounds of protein (peptide) character in RNA prepared by various methods and carefully freed from contaminations has also been found in other laboratories[10,11,14]. The finding of nucleotides associated with peptides in extracts from yeast[12] and rat liver[15] is very interesting. There is a possibility that these nucleotide-bound peptides have certain relations to RNA-bound peptides.

The peptide ingredient found in our laboratory may be an intermediary factor in the biosynthesis of proteins occurring on the surface of RNA, but it appears to be important also in the structure of the macromolecular RNA.

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New peptide-nucleotide compounds obtained from *Chlorella* and yeasts

Previous publications[1,2,3] reported that *Chlorella* cells contain a certain sulfur-containing substance(s) whose amount increases considerably when the cells enter the stage of nuclear division. This substance which is extractable with 10% TCA was found to contain a peptide-like substance(s) and nucleotide(s) as its components. Subsequent studies showed that an almost identical substance was obtainable from other organisms such as *Saccharomyces formosensis* and *S. cerevisiae*. In this paper, data will be presented which give more information as to the chemical nature of this substance.

Abbreviations: TCA, trichloroacetic acid; A, adenine; G, guanine; U, uracil.