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.

Institute of Medical Chemistry, Charles University Medical Faculty, Plzeň, Karlovarská 48 (Czechoslovakia) VLASTIMIL HABERMANN

1 A. GIERER AND G. SCHRAMM, *Nature*, 177 (1956) 702.
2 J. S. COLTER, H. H. BIRD AND R. A. BROWN, *Nature*, 179 (1957) 859.
3 E. WECKER AND W. SCHÄFER, *Z. Naturforsch.*, 12b (1957) 415.
4 PING-YAO CHENG, *Nature*, 181 (1958) 1800.
5 G. KOCH, H. E. ALEXANDER, I. M. MOUNTAIN, K. SPRUNT AND O. V. DAMME, *Federation Proc.*, 17 (1958) 257.
6 M. B. HOAGLAND, P. C. ZAMECNIK, M. L. STEPHENSON, *Biochim. Biophys. Acta*, 24 (1957) 215.
7 R. SCHWEET, E. GLASSMAN, E. ALLEN, *Federation Proc.*, 17 (1958) 307.
8 E. J. OFENGAND, F. H. BERGMANN AND P. BERG, *Federation Proc.*, 17 (1958) 284.
9 R. W. HOLLEY AND P. PROCK, *Federation Proc.*, 17 (1958) 244.
10 M. HOLDEN AND N. W. PIRIE, *Biochem. J.*, 60 (1955) 46.
11 L. K. RAMACHANDRAN AND H. FRAENKEL-CONRAT, *Arch. Biochem. Biophys.*, 74 (1958) 224.
12 V. V. KONINGSBERGER, CHR. O. VAN DER GRINTEN AND J. TH. G. OVERBEEK, *Biochim. Biophys. Acta*, 26 (1957) 483.
13 B. KEIL AND M. HRUBEŠOVÁ, *Chemické listy*, 49 (1955) 274.
14 H. FRAENKEL-CONRAT, B. SINGER AND R. C. WILLIAMS, *Biochim. Biophys. Acta*, 25 (1957) 87.
15 C. WEINSTEIN, D. HAMMOND, J. I. BERKMAN, P. M. GALLOP, S. SEIFTER, *Federation Proc.*, 17 (1958) 332.

Received November 25th, 1958

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.

Fresh ^{35}S-labeled cells of *Chlorella* or of yeast were directly extracted with cold 10 % TCA, and after the pH of the extract was adjusted to 7, an excess of ethanol (final conc., 90 %) was added. The precipitates were centrifuged, dried *in vacuo*, and after being dissolved in distilled water, subjected to zone electrophoresis and anion-exchange chromatography. Zone electrophoresis with starch as the medium showed that the substance in question did not move even at pH 1.2–2.3. In Fig. 1 are reproduced the results of anion-exchange chromatography (using Dowex-1-chloride) which were obtained with the materials from *Chlorella* and *Saccharomyces formosensis*.

While Fractions II and III were ninhydrin negative before hydrolysis, Fraction I showed a positive ninhydrin reaction even before hydrolysis, although the reaction increased considerably after hydrolysis.

By paper-chromatographic and spectrophotometric analyses of the hydrolysate (1 *N* HCl, 100°, 1 h; or trifluoroacetic acid, 155°, 80 min[4]) it was found that all fractions contained A, either free or combined to U, to U and G or to an unknown substance (X). The orcin-HCl and diphenylamine tests of the samples showed that all fractions contained a ribose-type sugar as a constituent. The similarity of the substances obtained from the two organisms was also notable in the composition of acid hydrolysates which were analysed by paper chromatography followed by autoradiography (see Table I). In both cases, Fraction I contained cyst(e)ine, glutamic acid, glycine, aspartic acid, serine and two unidentified S-compounds. Fraction II from yeast contained no arginine, but contained glycine, aspartic acid and an unidentified S-compound, which were lacking in Fraction II from *Chlorella*.

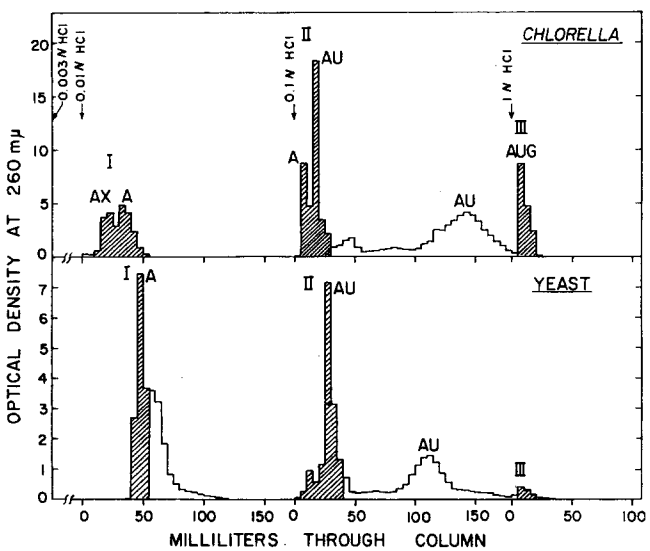

Fig. 1. Anion-exchange chromatograms of the TCA extract of *C. ellipsoidea* and of *S. formosensis*, as traced by the absorbance at 260 mμ. Exchanger: Dowex-1-chloride, 200–400 mesh, 13 cm × 1 cm^2. Eluants: 0.01 *N*, 0.1 *N* and 1.0 *N* HCl as indicated. Letters A, U, G and X signify that the fractions were found to contain these substances, when their hydrolysates were subjected to paper chromatography followed by spectrophotometric analyses. Shaded portions of polygons indicate that the eluates contained, besides u.v.-absorbing substances, ^{35}S and the substances which gave positive or increased ninhydrin reaction after hydrolysis with 6 *N* HCl at 100° for 14 h. The three main shaded polygons are referred to as Fractions I, II and III in the text.

TABLE I

AMINO ACIDS IN THE FRACTIONS SEPARATED BY ANION-EXCHANGE CHROMATOGRAPHY FROM THE TCA EXTRACTS OF *Chlorella* AND YEAST

The fractions were hydrolysed with acid and subjected to paper chromatography for identification of amino acids.

Fraction (eluant)	*I (0.01 N HCl)*	*II (0.1 N HCl)*	*III (1 N HCl)*
Chlorella (*C. ellipsoidea*)	Cyst(e)ine Two unidentified S-compounds* Glutamic acid Glycine Aspartic acid Serine	Cyst(e)ine Arginine Glutamic acid Alanine	Cyst(e)ine Glutamic acid Aspartic acid Serine An identified substance
Yeast (*S. formosensis*)	Cyst(e)ine Two unidentified S-compounds* Glutamic acid Glycine Aspartic acid Serine	Cyst(e)ine An unidentified S-compound** Glutamic acid Glycine Aspartic acid Alanine	—

* These S-containing substances appear to be the same in *Chlorella* and the yeast, judging from the data of paper chromatography.

** This substance seems to be identical with one of the two S-containing substances appearing in Fraction I.

Further zone-electrophoretic and paper-chromatographic experiments led us to the inference that in each fraction studied the amino acids and the unidentified substance(s) listed in Table I constitute a peptide or peptides which are bound to nucleotides or polynucleotides. These compounds might, at least partly, be identical with the compounds reported recently by KONINGSBERGER *et al.*[5] who found certain peptide-nucleotide compounds in the water extracts of baker's yeast frozen with ether and CO_2. The peptide–nucleotide compound found in our experiment might have originally been a single complex compound, which would have been split in smaller units in the process of chromatography. Clarification of this point as well as elucidation of the nature of the unidentified S-compounds and of substance X described above is a matter for further investigation.

EIJI HASE
SAYOKO MIHARA
HAMA OTSUKA
HIROSHI TAMIYA

The Institute of Applied Microbiology, University of Tokyo, and The Tokugawa Institute for Biological Research, Tokyo (Japan)

[1] E. HASE, Y. MORIMURA AND H. TAMIYA, *Arch. Biochem. Biophys.*, 69 (1957) 149.
[2] E. HASE, Y. MORIMURA, S. MIHARA AND H. TAMIYA, *Arch. Mikrobiol.*, 32 (1958) 87.
[3] E. HASE, H. OTSUKA, S. MIHARA AND H. TAMIYA, *Biochim. Biophys. Acta*, in the press.
[4] S. K. DUTTA, A. S. JONES AND M. STACEY, *J. Gen. Microbiol.*, 14 (1956) 160.
[5] V. V. KONINGSBERGER, CHR. O. VAN DER GRINTEN AND J. TH. G. OVERBEEK, *Biochim. Biophys. Acta*, 26 (1957) 490.

Received November 18th, 1958