Preliminary Notes

Distribution of guanylic acid in RNA of yeast and tobacco mosaic virus

Ribonuclease T_1 which specifically splits the internucleotide bonds between 3'-guanylic acid and 5'-hydroxyl groups of adjacent nucleotides was purified from takadiastase[1], and it was suggested as an useful tool for obtaining information on the nucleotide arrangement in RNA[2].

RNA was prepared from tobacco mosaic virus as a single molecular species by the phenyl method of GIERER AND SCHRAMM[3], followed by precipitation with 1 *M* NaCl[4]. For comparison, highly polymerized yeast RNA (preparation no. 9) was prepared by the use of sodium dodecyl sulfate according to CRESTFIELD *et al.*[5], although it is a heterogeneous polynucleotide mixture[6]. Commercial yeast RNA (Schwarz Laboratories) was purified by deproteinization and compared with high-molecular-weight RNA in regard to its behavior with RNase T_1.

RNA was mixed with RNase T_1 in Tris buffer (0.05 *M*, pH 7.5), and incubated at 37° for 6 h. The released mononucleotides consist of guanosine 3'-phosphate and guanosine 2',3'-cyclic phosphate. The cyclic nucleotide was converted to guanosine 2'-phosphate and guanosine 3'-phosphate by the incubation of the enzyme digest in 0.1 *N* HCl for 20 h at 2°. The resulting guanosine 2'-phosphate and guanosine 3'-phosphate were separated from other nucleotide components by paper chromatography with the Whatman No. 1 and the solvent[7]: satd. $(NH_4)_2SO_4$–water–isopropanol (79:19:2, v/v/v). Guanylic acid was eluted in 0.1 *N* HCl and determined spectrophotometrically. The amount of the guanine mononucleotide was expressed as per cent of the total guanylic acid in RNA and of the total nucleotides in RNA, based on phosphorus analysis in the digest. Nucleotide composition was determined as described previously[8].

TABLE I

NUCLEOTIDE COMPOSITION AND GUANINE MONONUCLEOTIDE IN RNASE T_1 DIGEST

RNA preparation	*Nucleotide ratio (mole %)*				*Guanine mononucleotide obtained by RNase T_1 digestion*		
					% of total guanylic acid		*mole % of total nucleotides*
	Guanylic	*Adenylic*	*Cytidylic*	*Uridylic*	*Calculated**	*Determined*	*Determined*
Yeast RNA (Schwarz)	29.0	25.8	20.4	24.8	29.0	50.5	14.6
Yeast RNA-9	27.5	25.6	20.5	26.4	27.5	48.7	12.7
TMV-RNA	24.0	28.0	19.2	28.8	24.0	55.7	13.4

* On basis of random distribution.

The results are shown in Table I. The amount of guanine mononucleotide in the RNase T_1 digest corresponds to the amount of guanylic acid clusters containing

Abbreviations: RNA, ribonucleic acid; TMV, tobacco mosaic virus; Tris, tris(hydroxymethyl) aminomethane.

two or more guanylic acid in RNA. In TMV-RNA, this value is larger than in yeast RNA, and both deviate significantly from the random distribution. In these RNA's guanine nucleotide tends to be next to guanine nucleotide. The RNase T_1 attacked the highly polymerized RNA as well as low-molecular-weight RNA.

Further studies on the nucleotide distribution in RNA with the combination of the use of RNase T_1, pancreatic RNase I, and other methods are now in progress.

Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Hongo, Tokyo (Japan)

KIN-ICHIRO MIURA*
FUJIO EGAMI

1 K. SATO AND F. EGAMI, *J. Biochem. (Tokyo)*, 44 (1957) 753; K. SATO-ASANO, *J. Biochem. (Tokyo)*, 46 (1959) 31.
2 K. SATO-ASANO AND F. EGAMI, *Nature*, 185 (1960) 462.
3 A. GIERER AND G. SCHRAMM, *Z. Naturforsch.*, 11b (1956) 138.
4 K. MIURA *et al.*, manuscript in preparation.
5 A. M. CRESTFIELD, K. C. SMITH AND F. W. ALLEN, *J. Biol. Chem.*, 216 (1955) 185.
6 K. MIURA, T. KITAMURA AND Y. KAWADE, *Biochim. Biophys. Acta*, 27 (1958) 421.
7 R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 49 (1951) 401.
8 K. MIURA AND K. SUZUKI, *Biochim. Biophys. Acta*, 22 (1956) 565.

* Visiting from the Institute for Virus Research, Kyoto University, Kyoto.

Received August 18th, 1960

Biochim. Biophys. Acta, 44 (1960) 378–379

Isolation of an enzyme catalyzing the transfer of amino acids from soluble RNA to microsomal ribonucleoprotein

In the course of incorporation of amino acids into microsomal RNP, recent investigations have demonstrated that activated amino acids formed a complex with a soluble RNA of relatively low molecular weight, and that the amino acids so bound to s-RNA were subsequently transferred to RNP[1,2]. Although it has been assumed that the latter transfer reaction is catalyzed by an enzyme which might be called "transferring enzyme" present in the cytoplasmic supernatant[2,3], isolation of such an enzyme has not yet been accomplished.

An approx. 15 % rat-liver homogenate was prepared in 0.25 *M* sucrose, 0.025 *M* KCl, 0.005 *M* $MgCl_2$, and 0.05 *M* Tris buffer, pH 7.6, and was centrifuged for 2 h at 105,000 × *g*. The supernatant fluid thus obtained was removed and its pH was lowered to 4.8–5.0 by 1 *N* acetic acid. The resulting precipitate was removed by a low-speed centrifugation (pH-5 fraction), and the pH of the supernatant was immediately adjusted again to pH 7.6 (pH-5 supernatant). Since the pH-5 supernatant showed a higher transferring activity than the pH-5 fraction and it does not catalyse activation of amino acids or overall incorporation of amino acids into RNP[2–4], the pH-5 supernatant was used for the isolation of the transferring enzyme. The method of purification of RNP from the microsome was the same as described in the preceding paper[5],

Abbreviations: RNA, ribonucleic acid; s-RNA, soluble RNA; RNP, ribonucleoprotein; GTP, ATP, CTP, and UTP, triphosphates of guanosine, adenosine, cytidine, and uridine; Tris, tris-(hydroxymethyl)aminomethane; DEAE-cellulose, diethyaminoethyl-cellulose.