Studies on synthesis of soluble ribonucleic acid. III. Analytical studies on soluble ribonucleic acid of rat liver

It has been shown[1,2] that the enzyme systems which incorporate ribonucleotide units into s-RNA can be fractionated from rat liver along with the s-RNA into 3 enzymically active ribonucleoproteins designated α, β, and γ. Each ribonucleoprotein can be fractionated further into the enzyme components and the s-RNA component[3]. The purpose of this report is to present analytical data on the s-RNA components fractionated from the parent ribonucleoproteins β and γ and on s-RNA prepared from the pH-5 enzyme fraction by extraction with phenol[4].

Analysis of alkaline hydrolysates of s-RNA β, s-RNA γ, and phenol-extracted s-RNA for their ribonucleoside content reveals the presence of adenosine, guanosine, cytidine, and uridine. From the quantities of these compounds given in the Table I,

TABLE I

COMPOSITION OF s-RNA β, s-RNA γ, AND s-RNA EXTRACTED FROM THE pH-5 ENZYME SYSTEM WITH PHENOL

10 mg each of s-RNA β and s-RNA γ isolated as described[3], and s-RNA extracted from the pH-5 enzyme fraction with phenol[4], all with $E_{250}:E_{280}$ ratios of 2.05–2.1, were hydrolysed in an excess of 0.1 *N* NaOH for 45 min at 85°. The hydrolysates were neutralized and then placed on Dowex-1 (200–400 mesh) formate columns (10 × 1 cm), and the elution was carried out by the method of COHN AND VOLKIN[10]. The ribonucleosides were eluted with 0.01 *N* HCOOH, concentrated to 10 ml *in vacuo*, and placed on Dowex-1 chloride columns (10 × 1 cm) in 0.01 *M* $K_2B_4O_7$. The elution was carried out according to the procedure described by COHN[11]. The u.v.-absorbing compounds in the fractions eluted from the columns were identified by comparing their positions in the chromatogram and their spectra in acid and alkali with those of authentic compounds. Pseudouridylic acid was identified by its position in the chromatogram and by the pronounced shift in the u.v. spectrum at 290 mμ between pH 11 and 12[12]. The compound listed as guanosine diphosphate has been identified, tentatively, as guanosine 3′(and 2′),5′-diphosphate on the basis of the following evidence: (1) The ratio guanine:ribose:phosphorus in the compound is 1.0:1.1:2.1; (2) the compound moves faster than guanosine 5′-diphosphate but slower than guanosine 5′-triphosphate upon electrophoresis on paper at pH 7.0; (3) under conditions known to result in the liberation of almost 1 mole of orthophosphate from 1 mole of guanosine 5′-diphosphate (10 min at 100° in 1.0 *N* HCl) no detectable orthophosphate is liberated from this compound.

	Ribonucleosides				
Component	*Adenosine*	*Uridine*	*Guanosine*	*Cytidine*	*Guanosine diphosphate**
s-RNA β	0.60	—	0.24	0.10	1.02
s-RNA γ	0.23	0.10	0.51	0.14	0.95
pH-5 s-RNA	0.67	0.12	0.14	0.07	1.21

	*2′,3′-Ribonucleotides**						
	AMP	*UMP*	*ψ-UMP*	*GMP*	*CMP*	*No of nucleotides per chain*	*Wt. of chain*
s-RNA β	7.2	6.0	1.01	13.0	13.2	42	15,000
s-RNA γ	6.0	5.0	1.21	11.1	11.0	36	13,800
pH-5 s-RNA	12.0	10.5	1.61	19.0	20.5	66	22,100

* Molar composition calculated by assuming that one terminal ribonucleotide residue corresponds to one polynucleotide chain.

Abbreviations: AMP, UMP, ψ-UMP, CMP, GMP, the mixed 2′-, 3′-isomers of adenylic, uridylic, pseudouridylic, cytidylic and guanylic acids, respectively; s-RNA, soluble ribonucleic acid.

it appears that most of the polynucleotide chains terminate with either adenylic acid or guanylic acid and that the proportion of chains which terminate in cytidylic acid is similar in all cases. The analysis of the alkaline hydrolysate also reveals the presence of guanosine 3′ (and 2′),5′-diphosphate in molar amounts equivalent to the sum of the ribonucleosides. This suggests that the ribonucleotide at the other end of all the chains is guanylic acid. Similar findings have been reported for s-RNA isolated from rabbit liver by a salt-extraction method[5] and for s-RNA isolated from *Escherichia coli* by a phenol-extraction method[6].

The determination of the average number of ribonucleotides per polynucleotide chain from the data in Table I gives values of 42 nucleotides for s-RNA β, 36 nucleotides for s-RNA γ, and 66 nucleotides for s-RNA extracted from the pH-5 enzyme fraction with phenol. These values correspond to chain weights of 15,000, 12,700, and 22,100 respectively. The chain weights of s-RNA β and γ are consistent with observed sedimentation coefficients ($S_{20,w}$) of 1.85 S[13].

Analysis of the hydrolysates for their ribonucleotide content shows that the ratio of pseudouridylic acid (ψ-UMP)[7,8] plus uridylic acid to adenylic acid and of cytidylic acid to guanylic acid is close to 1. Base ratios of this kind appear to be characteristic of the composition of s-RNA isolated from a wide variety of sources[5,6,9]. These findings are suggestive that base pairing may be of considerable importance in the structure and biological properties of s-RNA.

The results presented in this paper and studies on the physical characteristics of these s-RNA molecules will be discussed more fully in a later publication.

Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass. (U.S.A.) EDWARD HERBERT

Department of Pharmacology, Yale University School of Medicine, New Haven, Conn. (U.S.A.) E. S. CANELLAKIS

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