Studies on the fractionation of ribonucleic acid

As first shown in this laboratory, DNA can be separated into a series of fractions of regularly graded composition through the fractional dissociation of native or artificially prepared nucleic acid–histone complexes by salt solutions of increasing strength in the presence of a $CHCl_3$ phase[1,2]. The present note explores the application of this method to RNA.

RNA specimens (free of DNA) were prepared from rat liver by a modification of a previously described procedure[3]. The tissue was ground for 1 min in 5 vol. 10^{-1} *M* EDTA, pH 8, and the pulp treated with an equal vol. of 90% phenol for 1 h at room temp. The aqueous phase, separated by centrifugation (20,000 × *g*, 30 min) and combined with the EDTA washings of the phenol layer, was adjusted to a 2% potassium acetate concentration and mixed with 2 vol. ethanol. The RNA thus precipitated was dissolved in EDTA and separated into high-molecular RNA insoluble in 10% NaCl (about 80% of the total) and into RNA soluble in strong salt solution (15–20%).

Conversion to the respective histone nucleates was followed by their stepwise extraction, in the presence of $CHCl_3$, with increasing concentrations of NaBr under the conditions otherwise specified previously[2]. The insoluble RNA thus was divided into 8 fractions between 0.5 and 5.2 *M* NaBr, the soluble RNA into 5 fractions between 0.2 and 1.0 *M* NaBr.

The general procedure for the estimation of the nucleotide composition of the fractions has been described[1]; but it appeared of interest also to study the distribution of minor RNA constituents, especially of pseudouridylic acid. For the quantitative determination of the latter, alkaline hydrolysates were subjected to descending 2-dimensional paper chromatography: I, isopropanol–NH_3 [5](72 h); II, isobutyric acid–ammonium isobutyrate[6] (20 h). In I, pseudo uridylic precedes guanylic acid, but is slower than the band containing the other nucleotides; in II, uridylic and guanylic acids run adjacent to each other, whereas pseudouridylic acid is slightly slower. All 5 constituents can thus be separated. The identity of pseudouridylic acid was established by chromatographic comparison with an authentic sample kindly supplied by Dr. W. E. Cohn[7] and also through the characteristic spectra at pH 1 and 13[8]. The nature of several minor components also seen on the chromatograms is being investigated.

As concerns the nucleotide composition of the RNA specimens before fractionation, Table I suggests the existence of substantial differences between the RNA insoluble in 10% NaCl and the soluble RNA, the former exhibiting higher proportions of guanylic and cytidylic acids. The most striking divergence is seen in the proportion of pseudouridylic acid which is much higher in the soluble RNA. This finding is similar to observations on pseudouridine in the microsomal and the non-sedimentable RNA of rat liver[9]. By way of comparison, we have found the NaCl-insoluble RNA isolated from a thymine-requiring strain of *Escherichia coli* to be free of trace components.

Of particular interest are the differences between DNA and RNA as regards the fractional dissociation, under denaturing conditions, of their respective histone

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; EDTA, ethylenediaminetetraacetate.

TABLE I

INSOLUBLE AND SOLUBLE RAT LIVER RNA; COMPOSITION AND FRACTIONATION*

Starting preparation	Fraction			Moles/100 moles nucleotide in RNA					Molar ratios		
	No.	NaBr (M)	% of RNA in starting material	A	G	C	U	Ps	$\frac{A+U+Ps}{G+C}$	$\frac{6\text{-}Am}{6\text{-}K}$	$\frac{Ps}{U}\times 100$
Insoluble RNA	Total	—	100	19.6	32.2	29.7	17.3	1.2	0.62	0.97	6.9
	1	0.5	4.0	21.7	29.7	26.8	19.5	2.2	0.77	0.94	11.3
	2–8	0.65–5.2	82.3	18.6–19.5	32.1–33.7	29.0–31.1	16.2–17.9	1.0–1.3	0.59	0.96	7.0
Soluble RNA	Total	—	100	20.5	30.4	28.3	17.4	3.3	0.70	0.95	19.0
	1	0.2	33.0	21.2	29.8	29.0	16.3	3.8	0.70	1.01	23.3
	2–4	0.35–0.75	62.5	21.0–21.4	30.4–31.4	27.2–28.9	15.2–18.3	3.1–3.4	0.69	0.96	19.0
	5	1.0	3.9	21.3	30.8	28.2	17.9	1.8	0.69	0.98	10.0

* A, G, C, U, Ps stand for adenylic, guanylic, cytidylic, uridylic and pseudouridylic acids, respectively. 6-Am denotes the 6-amino nucleotides (A + C), 6-K the 6-keto nucleotides (G + U + Ps). For the sake of brevity, only the range of analytical results is given for Fractions 2–8 of the insoluble RNA and for Fractions 2–4 of the soluble RNA; in these cases the molar ratios represent averages. For the quantitative estimation of Ps, a value of $\varepsilon = 8{,}400$ at 262.5 mμ in 0.1 N HCl was used[8].

nucleates. Not only is the RNA, especially the portion insoluble in salt, more strongly held by histone, requiring, for dissociation, the more effective bromide rather than chloride, but the various fractions, although released characteristically over a wide molarity range, appear to differ less in composition than is observed with DNA. It will be seen in Table I that—in contrast to the DNA fractions exhibiting a gradual transition from the GC to the AT type[1,2]—only Fraction 1 of the insoluble RNA was significantly different in composition from that of the total in having a higher content of adenylic, uridylic and pseudouridylic acids. The 5 fractions yielded by the soluble RNA differed mainly with regard to the concentration of pseudouridylic acid, especially when Fractions 1 and 5 are compared. In all cases, the ratio 6-Am/6-K[10] was close to unity. It should be added that there is some evidence that the fractions, despite similarities in their composition, are distinguished by certain features of the nucleotide arrangement. This, as well as related investigations, will be discussed later in another context.

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Effect of magnesium on the amino acid-dependent pyrophosphate exchange catalysed by "RNA-low" systems

Recent evidence suggests that the stability of the leucine-activating enzyme present in rat-liver pH-5.0 fractions[2], is due to a special association between enzyme and "soluble" RNA, conferring protection upon labile thiol groups concerned both in pyrophosphate exchange, and in the transfer of [^{14}C]leucine to "soluble" RNA. The instability of activating enzymes found in "RNA-low fractions" obtained from protamine-treated rat-liver extracts[1,3], probably results from the interaction or

Abbreviations: RNA, ribonucleic acid; ATP, adenosine triphosphate; AMP, adenosine 5′-phosphate; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol.