Differential Stability of 28s and 18s Rat Liver Ribosomal Ribonucleic Acids

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Rat liver ribosomal RNA (rRNA) free from nuclease contaminants was isolated by a modification of the phenol technique. The 28s and 18s rRNA species were separated by preparative agar-gel electrophoresis. The two rRNA species were heated at different temperatures under various conditions and the amount of undegraded rRNA was determined by analytical agar-gel electrophoresis. The 18s rRNA remained unaltered after heating for up to 10min. at 90° in water, acetate buffer, pH5·0, or phosphate buffer, pH7·0. Under similar or milder conditions 28s rRNA was partially degraded, giving rise to a well-delimited 6s peak and a heterogeneous material located in the zone between 28s and 6s. The dependence of degradation of 28s rRNA on the temperature and the ionic strength of the medium was studied. The greatest extent of degradation of 28s rRNA was observed on heating at 90° in water. It is suggested that the instability of rat liver 28s rRNA is due to two factors: the presence of hidden breaks in the polymer chain and a higher susceptibility of some phosphodiester bonds to thermal hydrolysis.

It is generally presumed that each of the two rRNA* species is a continuous polynucleotide chain (see Spirin & Gavrilova, 1968; Cox, 1968). However, with rRNA of animal cells it has been shown in several cases that the 28s component is not intact even when isolated under conditions in which degradation during the preparative steps is highly unlikely (Petermann & Pavlovec, 1963; Tsanev, 1965; Applebaum, Ebstein & Wyatt, 1966; Venkov & Hadjiolov, 1967). Thus the existence of some 'hidden breaks' in the native 28s rRNA molecule cannot be safely ruled out. The presence of specific 'weak' points along the polynucleotide chains, rapidly hydrolysed during processing of rRNA, may be also envisaged.

In the present work with rat liver rRNA we tried to discriminate between the three main factors likely to affect the integrity and stability of rRNA: (*a*) the presence of endonuclease contaminants in rRNA preparations; (*b*) the thermal hydrolysis of phosphodiester bonds observed in aqueous solutions (Boedtker, Möller & Klemperer, 1962); (*c*) the existence in rRNA of some 'hidden breaks' in the polymer chain. The better resolving power of agar-gel electrophoresis (Tsanev, 1965; Hadjiolov, Venkov & Tsanev, 1966) was exploited to obtain a more detailed quantitative evaluation.

* Abbreviation: rRNA, ribosomal RNA.

MATERIALS AND METHODS

Rat liver cytoplasmic RNA was isolated and purified as described by Hadjiolov, Venkov, Dolapchiev & Genchev (1967). To remove any traces of nucleases present in the rRNA preparations, the solution of rRNA in 5mM-sodium acetate buffer, pH5·8, was passed through a column of Dowex 50 (Na⁺ form) and the rRNA collected in 96% (v/v) ethanol containing potassium acetate (1%, w/v) at 4°. The 28s and 18s rRNA species were separated by a preparative modification of the agar-gel electrophoresis technique of Tsanev & Staynov (1964). About 2mg. of rRNA was fractionated in a single run on 24cm. × 18·5cm. agar-gel plates under the standard conditions. The positions of the rRNA components were detected under direct u.v. light (low-pressure mercury lamp; Original-Hanau PL320) and the corresponding agar-gel bands were excised. The agar-gel bands were transferred separately to a suitable electrophoresis apparatus, the appropriate rRNA species was eluted from the agar gel by a short electrophoresis run (about 30–40min.) and collected in a dialysis bag interposed between the anode and the agar-gel band. The 28s and 18s rRNA fractions were precipitated with ethanol-potassium acetate as above. All the solutions and materials were previously sterilized and the manipulations carried out under sterile conditions. The yield of the two rRNA components was approx. 70–80%.

The heat treatment of rRNA solutions was carried out in stoppered test tubes, at a concentration of 17 E_{260} units/ml. for 28s rRNA and 10 E_{260} units/ml. for 18s rRNA in a volume of 0·5ml. After the heating step the tubes were

cooled rapidly and 0·06ml. samples analysed by the standard agar-gel-electrophoresis technique (Tsanev & Staynov, 1964). A control sample was run parallel to each experimental sample of RNA to obtain a better quantitative evaluation of the results. The dried agar-gel electrophoretograms were scanned at 260nm. with an RSD 220 recording spectrodensitometer (Electroimpex, Sofia, Bulgaria). The area of each rRNA peak was determined with a planimeter or calculated as the area of an isosceles triangle. The amount of undegraded rRNA after the heat treatment was related to the area of the control peak taken as 100. The accuracy of this technique determined from 40 independent runs of a 28s rRNA sample is ± 3·0%.

RESULTS

General pattern of degradation of rRNA. Incubation of the rRNA preparations at 37° was taken as a criterion for the absence of nuclease contaminants. As shown in Fig. 1, incubation of purified rRNA at 37° for 24hr. does not cause any significant change in the shape of the 28s and 18s rRNA peaks and such preparations are considered virtually free of nucleases. As shown in Fig. 1(d), incubation at 37° for 8 days causes some degradation of 28s rRNA (and perhaps of 18s rRNA), revealed by the shoulder on the right of the 28s rRNA peak. However, even after 8 days the two rRNA peaks are well delimited and coincide in position with those of the original rRNA preparation.

Heating of rRNA at 90° results in the partial degradation of 28s rRNA, whereas the 18s rRNA remains apparently unchanged. The degradation products released from rRNA on heating were investigated with the separate 28s (Figs. 1e and 1f) and 18s (Figs. 1g and 1h) rRNA components. Degradation of 28s rRNA is accompanied by the formation of a definite peak at about 5–6s, with the remaining material heterogeneously distributed in the zone between 28s and 6s. It should be noted that the undegraded 28s rRNA preserves the mobility in agar gel typical of native 28s rRNA.

Temperature- and pH-*dependence of degradation of rRNA.* Samples of 28s and 18s rRNA were dissolved in sodium acetate buffer, pH5·0 and I 0·01, and treated for 60sec. at different temperatures. Parallel samples were heated in the presence of phenol saturated with the same buffer. The results given in Fig. 2 indicate that degradation of 28s rRNA shows a linear dependence on temperature under conditions at which 18s rRNA remains unaltered. Heating in the presence of phenol does not change the extent of degradation of 28s rRNA and therefore it seems unlikely to be due to nuclease contaminants.

Experiments were carried out in which 28s rRNA was heated for different times in sodium acetate buffer, pH5·0 and I 0·02, or sodium phosphate buffer, pH7·0 and I 0·03. The results
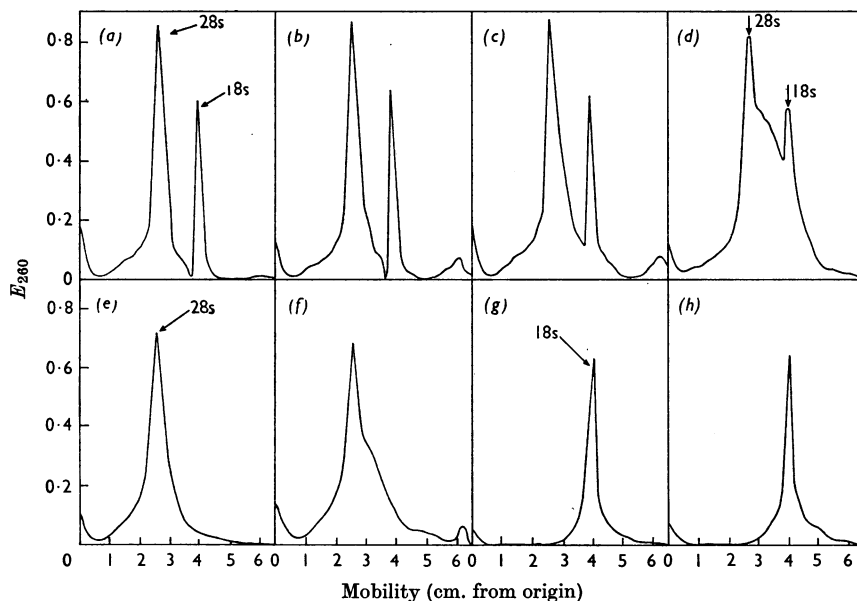

Fig. 1. Agar-gel-electrophoresis pattern of rat liver rRNA under different treatments. (a)–(d), Total rRNA incubated at 37° for different times: (a) 0hr.; (b) 4hr.; (c) 25hr.; (d) 8 days. (e) and (f), Isolated 28s rRNA, before (e) and after (f) heating for 120sec. at 90°. (g) and (h), Isolated 18s rRNA, before (g) and after (h) heating for 120sec. at 90°. The dried electrophoretograms were scanned at 260nm. For details see the text.
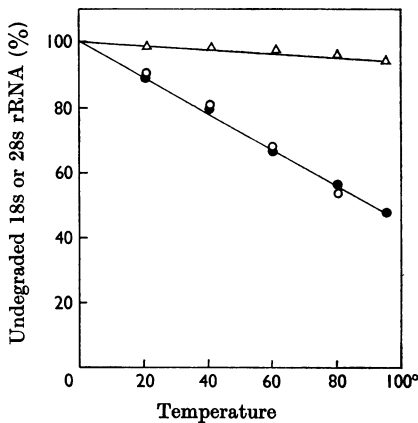
Fig. 2. Degradation of 28s (○ and ●) and 18s (△) rRNA dissolved in acetate buffer, pH 5·0, on heating at different temperatures for 60 sec. The area of the rRNA peak in the controls is taken as 100.
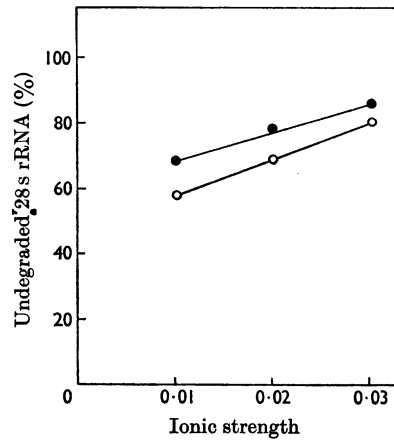

Fig. 3. Effect of ionic strength of the medium on the degradation of 28s rRNA at pH 5·0 (○) and pH 7·0 (●) on heating at 93° for 60 sec. Other designations are as in Fig. 2.

Table 1. *Degradation of rat liver* 28s *rRNA by heat treatment*

Samples of 28s rRNA in acetate buffer, pH 5·0, or in phosphate buffer, pH 7·0, were heated for different times at the specified temperatures. After immediate rapid cooling to 4°, rRNA was analysed by agar-gel electrophoresis. The amount of undegraded rRNA was determined by planimetry of the spectrodensitometer tracing at 260 nm. and referred to the area of the control sample taken as 100. For details see the text.

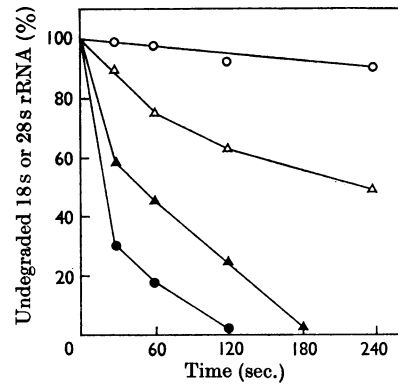| Duration of heat treatment (sec.)	Undegraded 28s rRNA (%)							
	Acetate buffer				Phosphate buffer			
	40°	60°	80°	90°	40°	60°	80°	90°
30	100	88	87	77	99	96	93	91
60	100	82	76	74	—	94	91	83
120	99	81	75	73	98	94	92	82
240	99	81	72	70	97	92	90	79
480	99	78	71	68	98	92	89	76

Fig. 4. Degradation of 28s rRNA dissolved in glass-distilled water on heating at 60° (△), 80° (▲) and 90° (●) and of 18s rRNA heated at 90° (○). Other designations are as in Fig. 2.

are presented in Table 1. These experiments revealed that a rapid partial degradation of 28s rRNA takes place in the first minute followed by a markedly lower rate in the subsequent 7 min. Differences in the extent of degradation of 28s rRNA at the two pH values tested are small. Nevertheless, these results indicate that the stability of 28s rRNA at pH 7·0 is higher than at pH 5·0.

Ionic strength and degradation of rRNA. The dependence of the stability of 28s rRNA on the ionic strength of the medium was studied by heating the sample at 93° for 60 sec. in sodium phosphate buffer, pH 5·0 or 7·0. The results given in Fig. 3 show a rather strong influence of the ionic strength

on degradation of 28s rRNA. The RNA molecule is stabilized at higher ionic strength.

Samples of 28s or 18s rRNA were also dissolved in glass-distilled water and heated at 60°, 80° or 90° for different times (Fig. 4). In this case degradation of rRNA is most clear-cut, complete degradation being attained on heating for 2 min. at 90° or for 4 min. at 80°. It should be noted that 18s rRNA remains stable under these conditions.

To avoid interference by thermal degradation, attempts were made to unravel 'hidden breaks' in rRNA by treatment with acid (Dr R. A. Cox, personal communication). Samples of rRNA in glass-distilled water were treated with an equal

volume of 10mM-hydrochloric acid for 10–45sec., neutralized with 63mM-sodium phosphate buffer, pH 7·0, and analysed by agar-gel electrophoresis. Under these conditions 18s rRNA remains unaltered, whereas 32% of the 28s rRNA is degraded to heterogeneous faster-moving material.

DISCUSSION

The present results indicate that rat liver 28s rRNA is less stable than 18s rRNA under various experimental conditions. The possibility that the observed degradation of 28s rRNA is due to nuclease contaminants present in the rRNA preparations studied may be safely eliminated. It is likely that rat liver 28s rRNA contains some 'hidden breaks' in the polynucleotide chains, whereas 18s rRNA chains are intact. These results are in agreement with chemical estimations of the chain lengths of rat liver 28s and 18s rRNA (Russev & Tsanev, 1969). The conditions used for the isolation of the rRNA preparations (direct homogenization of the tissue in phenol) make it highly unlikely that these 'hidden breaks' in the 28s rRNA molecule are obtained during its isolation and storage. Therefore the possibility that their presence reflects an initial stage in the process of breakdown of ribosomes and rRNA *in vivo* (Hadjiolov, 1966) should also be envisaged. Further, the polynucleotide chain of 18s rRNA appears also to be more resistant to thermal

hydrolysis than is that of 28s rRNA. This factor would explain the rapid degradation of 28s rRNA on heating of its solutions in distilled water. The reason for a preferential, presumably thermal, breakdown of phosphodiester bonds in 28s rRNA is not obvious (Boedtker *et al.* 1962). The exact quantitative evaluation of the contribution of these two factors in the degradation of rRNA requires further studies.

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