COMPARATIVE AGAR GEL ELECTROPHORESIS OF RNA, RIBOSOMES AND RIBOSOMAL SUBUNITS OF HIGHER EUKARYOTIC ORGANISMS

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1. Introduction

Polyacrylamide gel electrophoresis has been used to show that the ribosomal RNAs (rRNA) of the large ribosomal subunits of some higher organisms significantly vary with respect to their molecular weights [1]. Differences at the level of rRNA precursors were also detected [2, 3].

A systematic comparative study of rRNA, ribosomes and their subunits may be of great importance in elucidating the evolution of ribosomal structure.

In this study evidence is presented that ribosomes and their large subunits from eight different species of vertebrates differ in their electrophoretic mobilities which mainly reflects differences in the mobility of the RNA in the large ribosomal subunit. The mobility of the small ribosomal subunit and of its RNA exhibits no variations in the species studied.

2. Materials and methods

Livers of the following species were used: albino rat, chick, dove (*Columbia livia* var. *domestica*), water snake (*Natrix natrix*), horned viper (*Vipera ammodytes*), frog (*Rana ridibunda*), triton (*Triturus vulgaris*) and carp (*Cyprinus carpio*).

RNA was extracted from the total liver homogenates at 0° with water-saturated phenol pH 6.0 as described earlier for rat liver [4] and was carefully deproteinized [5].

'Crude' and 'purified' ribosomes were isolated [6] but no differences in their relative electrophoretic mobility were observed. In the case of frog and carp the ribosome pellet consisted of two layers: lower (gelatinous and colorless) and upper (yellow-brown). The upper layer was gently removed and used in these experiments.

Dissociation of ribosomes to subunits was achieved by the use of 0.002 M EDTA in 0.02 M tris-HCl pH 7.6 [6].

Rat liver rRNA and ribosomes were labelled in vivo for 48 hr by intraperitoneal injection of 50 μCi of ^{14}C-orotic acid (14.86 mCi/mmole).

RNA, ribosomes and their subunits were fractionated by agar gel electrophoresis [7, 8]. For comparative study agar gel slabs with six parellel slots were used. In some experiments labelled rat liver RNA was mixed with RNA of different origin.

The electrophoretograms and their radioautographs were scanned at 260 nm and 550 nm, respectively [9].

The rough values of sedimentation constants of unknown RNAs were determined graphically from their electrophoretic mobilities using as reference peaks rat liver 28 S, 18 S and 4 S RNAs [10].

The molecular weights were calculated from the corresponding 'S' values by the formula [11]

$$MW = 1550 \times S^{2.1}.$$

3. Results and discussion

Agar gel electrophoretic patterns of total liver RNAs from six of the species studied are shown in figs. 1 and 2. It is clearly seen that the electrophoretic mobility of the larger rRNA component is different whereas the electrophoretic mobility of the small size rRNA remains the same.

The electrophoretic mobility of the larger rRNA components gradually decreases as follows: triton >

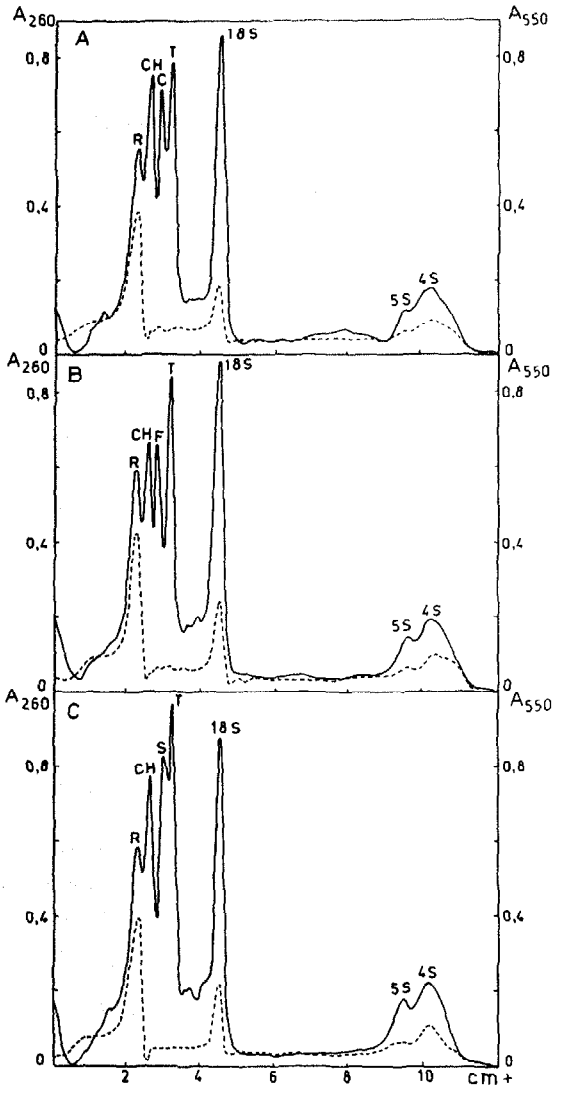

Fig. 1. Electrophoretic patterns of mixtures of total liver RNAs of different species. (——) absorbance at 260 nm; (- - - -) absorbance of radioautographs at 550 nm for rat liver RNA. R = rat; CH = chick; C = carp; T = triton, F = frog; S = snake. Conditions of electrophoresis: 2.0% agar, 0.02 M phosphate–citric acid buffer pH 8.0, containing 0.001 M of EDTA, 6.0 V/cm, 180 min, 15°.

snake > carp > frog > chick > rat. The calculated 'S' values and molecular weights for the larger rRNA component are summarized in table 1.

It is interesting to note that essentially the same differences in the electrophoretic mobility were

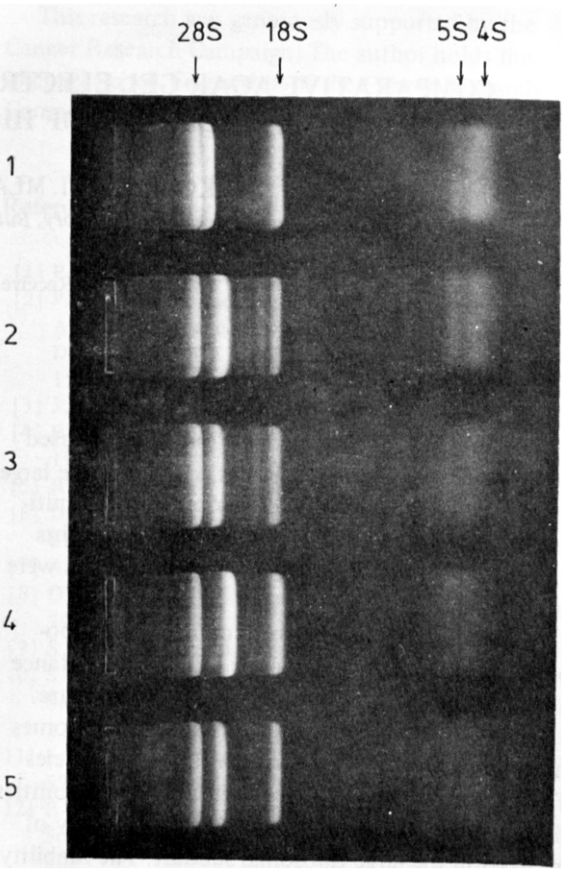

Fig. 2. Photoprint of electrophoretic patterns of total liver RNAs mixed with rat liver RNA. (1) chick; (2) snake; (3) frog; (4) triton; (5) carp. Conditions of electrophoresis as in fig. 1.

Table 1

Size of the large rRNA components in some vertebrates. 'S' represent the mean values determined graphically from 15–20 experiments. MW calculated from the corresponding 'S' values as indicated in Methods.

Species	'S'	MW(× 10^6)
Albino rat	28.0	1.69–1.70
Chick	26.5	1.51
Dove (*Columba livia*)	25.0	1.33
Water snake (*Natrix natrix*)	24.5	1.28
Horned viper (*Vipera ammodytes*)	24.5	1.28
Frog (*Rana ridibunda*)	25.7	1.41
Triton (*Triturus vulgaris*)	23.7	1.19
Carp (*Cyprinus carpio*)	25.0	1.33

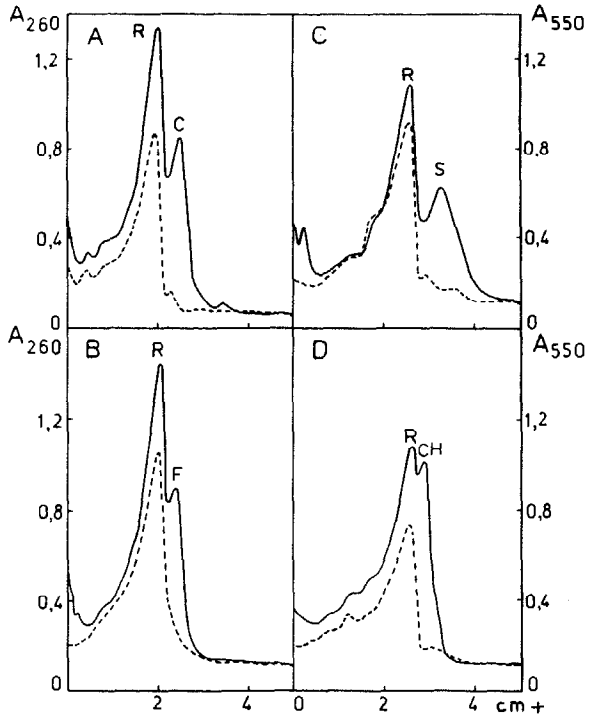

Fig. 3. Electrophoretic patterns of unlabelled ribosomes from different species mixed with labelled rat liver ribosomes. (——) absorbance at 260 nm; (- - - - -) absorbance of radioautographs at 550 nm. (A) carp; (B) frog; (C) snake; (D) chick. Conditions of electrophoresis: 1.25% agar, 0.02 M tris-HCl buffer pH 8.0, 6.3 V/cm, 165 min, 15°.

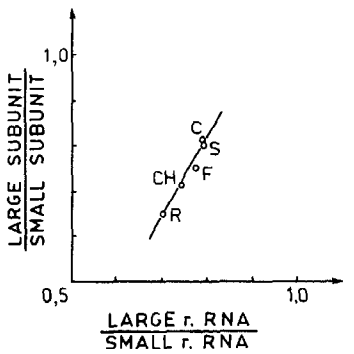

Fig. 5. Relationship between relative electrophoretic mobility of large ribosomal subunits and their corresponding rRNA. R = rat; CH = chick; C = carp; S = snake; F = frog.

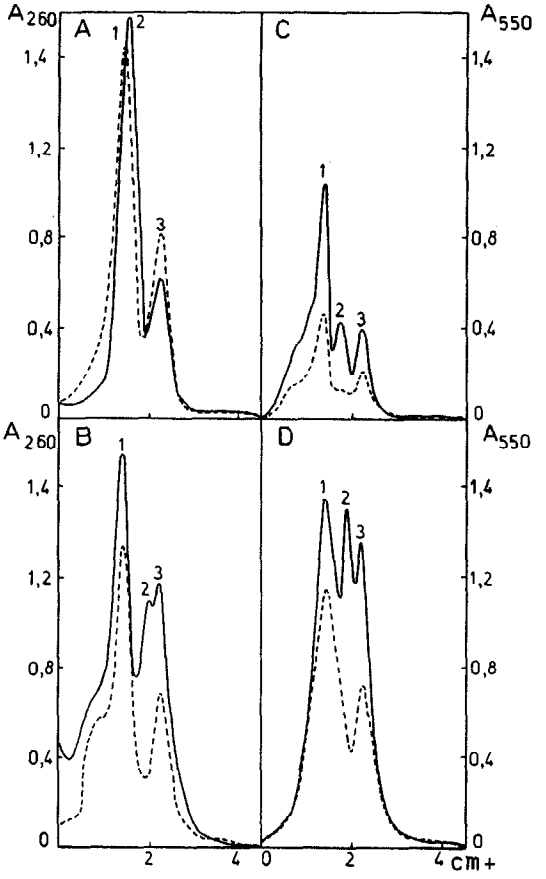

Fig. 4. Electrophoretic patterns of large and small ribosomal subunits. (——) absorbance at 260 nm; (- - - - -) absorbance of rat liver radioautographs at 550 nm. (A) labelled rat liver and unlabelled chick ribosomal subunits in parallel run; (B) mixture of snake and rat liver ribosomes, treated with EDTA; (C) mixture of frog and rat liver ribosomes, treated with EDTA. (D) mixture of carp and rat liver ribosomes, treated with EDTA. (1) large ribosomal subunit (rat); (2) large ribosomal subunit: chick (A), snake (B), frog (C) and carp (D); (3) small ribosomal subunit: rat and chick (A), rat and snake (B), rat and frog (C), rat and carp (D). Conditions of electrophoresis as in fig. 3.

detected for the corresponding ribosomes and their large subunits (figs. 3 and 4). The radioactivity profiles presented in fig. 3 show that there is no interaction between different kinds of ribosomes. Fig. 4 demonstrates that the small ribosomal subunits from all species studied have the same electrophoretic mobility.

On the basis of the parallelism in the electrophoretic

mobility of ribosomes and their large subunits, on the one hand, and the large rRNAs, on the other, it seems that differences in mobility of ribosomes and their large subunits are determined predominantly by the rRNA component in the large subunit. As seen in fig. 5 the relative electrophoretic mobility of different large subunits is roughly proportional to the relative electrophoretic mobility of the corresponding rRNA.

Our results indicate that some differences in the sedimentation of ribosomes and ribosomal subunits reported in the literature for different species [12, 13] may be due not only to differences in the conditions used but may reflect real differences in the size of the large ribosomal subunit.

It would be difficult at present to explain why evolutionary changes in the size are observed only in the large ribosomal subunit while the small subunit remains constant. Evidently some restrictions in the variation of the small subunit are imposed in connection with its specific function in the process of translation.

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