TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS SEPARATION OF LOW MOLECULAR WEIGHT NUCLEAR RNA

Ramachandra Reddy, Thomas O. Sitz, Tae Suk Ro-Choi and Harris Busch

Department of Pharmacology, Baylor College of Medicine

Houston, Texas 77025

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SUMMARY

Two-dimensional electrophoresis successively on 10%, pH 7.2, and 12%, pH 3.3, polyacrylamide gels was used to separate nuclear and whole cell 4-8S RNA fractions from Novikoff hepatoma ascites cells into individual RNA species. With this method, analytical studies were possible on two new species of RNA referred to as 4.2S RNA and 4.5S RNA_{II}, respectively. The 4.2S RNA has the nucleotide composition AMP, 18.0; UMP, 23.9; GMP, 27.9; and CMP 26.7. This RNA contains 2 ψMP residues and an alkali stable dinucleotide. The 4.5S RNA_{II} was distinctly separated from 4.5S RNA_{I} and 4.5S RNA_{III} and has the nucleotide composition AMP, 20.6%; UMP, 23.7%; GMP, 30.0%; and CMP, 25.7%. It has no ψMP or 2'-O-methylated nucleotides. In addition, several other spots were separated from the major RNA species.

INTRODUCTION

Single dimensional polyacrylamide gel electrophoresis has been extensively used in this and other laboratories to fractionate individual RNA species (1). Recently, two-dimensional polyacrylamide gels have been used to achieve greater resolution of proteins (2), RNA and partial digests of RNA (3) and transfer RNA (4). Earlier studies from this laboratory have shown that nuclear 4.5S RNA and 5S RNA obtained from preparative polyacrylamide gel electrophoresis were separated into several components on DEAE-Sephadex columns (5,6). The two-dimensional polyacrylamide gel electrophoresis method presented here provides a more satisfactory separation of these RNA species and, in addition, several new minor RNA species were found.

MATERIALS AND METHODS

Labeling of Nuclear RNA by ^{32}P In Vitro - The procedures for incubation of Novikoff hepatoma ascites cells with ^{32}P-orthophosphate, extraction of RNA by the SDS-phenol procedure and separation of 4-8S RNA fraction from high molecular weight RNA by sucrose density gradient have been described earlier (5).

Separation of 4-8S RNA Fraction by Two-Dimensional Acrylamide Gels - The 10% polyacrylamide gels (1:39, bisacrylamide:acrylamide, w/w) were made as 40 x 20 x 0.3 cm slabs (1). The buffer used contained 0.04 M Tris, 0.002 M EDTA, 0.02 M sodium acetate, pH 7.2. Slots 0.5 cm wide were made in the gel, and highly labeled 4-8S RNA was placed in the slot in 5% sucrose. The bromphenol blue marker was allowed to migrate 30-35 cm from the point of application, at 40 mA per gel at 4°C. After the first dimension run, the RNA bands were identified by autoradiography.

The second dimension was run at pH 3.3 in 6 M urea - 0.025 M citric acid with an acrylamide concentration of 12% (1:39, bisacrylamide:acrylamide, w/w). The second dimension gels, 40 x 40 x 0.3 cm, readily accommodated the long strips obtained from the first dimension. The gel strips from the first dimension were positioned between two clean glass plates before pouring the acrylamide solution. Soaking the acrylamide strip from the first dimension in the 6 M urea-citric acid pH 3.3 buffer for 15 min improved the polymerization around the strip. The acid gel was kept at room temperature or in the cold room overnight before starting electrophoresis. The 40 cm gel slabs were subjected to electrophoresis at 40 mA until the bromphenol blue marker migrates 30-35 cm. The RNA spots were identified by autoradiography.

Extraction and Nucleotide Composition of RNA - RNA was extracted and precipitated from the acrylamide gel as described

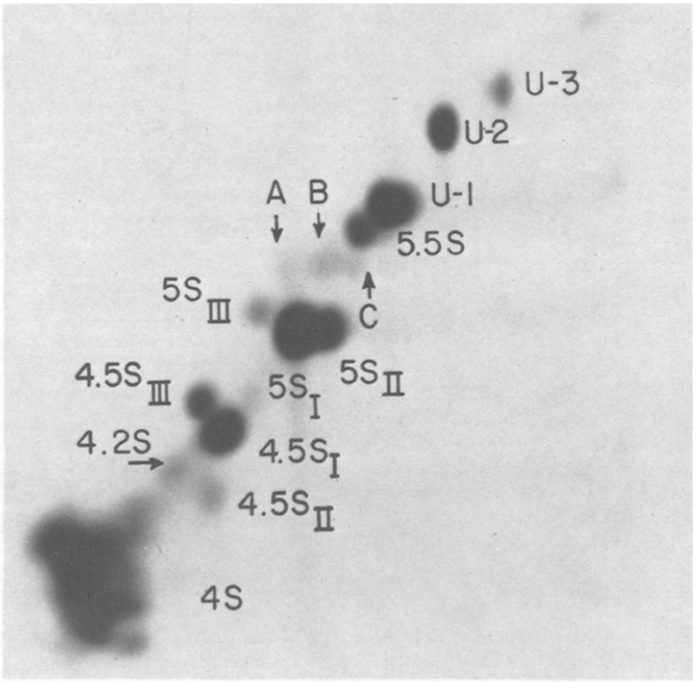

Fig. 1. Autoradiograph of 4-8S nuclear RNA separated by two-dimensional polyacrylamide gel electrophoresis. The first dimension gel contained 10% polyacrylamide and was run for 40 hr in a buffer containing 0.04 M Tris-acetate, 0.002 M EDTA, and 0.02 M sodium acetate, pH 7.2; the second dimension gel contained 12% polyacrylamide and was run for 40 hr at pH 3.3 in 0.025 M citric acid containing 6 M urea.

earlier (7). The air-dried RNA was hydrolyzed with 0.25 N NaOH for 16-18 hr at 37°C and the nucleotide compositions, the content of ψMP and 2'-O-methylated nucleotides were determined by electrophoresis at pH 3.5 in the first dimension and chromatography in the second dimension (8,9).

RESULTS

Separation of 4.5S RNA - Although the 4.5S RNA's were separated earlier on DEAE-Sephadex A-50 columns (5), the 4.5S RNA_{II} did not separate as a discrete peak. However, the two-dimensional procedure separated 4.5S RNA_{II} as a distinct spot (Fig. 1) which like 4.5S RNA_{I} (10) does not contain ψMP or 2'-O-methylated

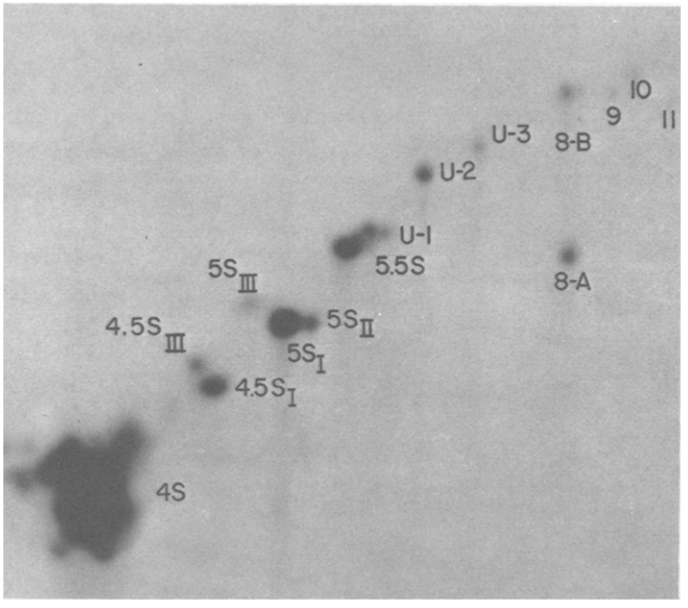

Fig. 2. Autoradiograph of 4-8S whole cell RNA separated by two-dimensional polyacrylamide gel electrophoresis. The conditions were the same as described for Figure 1.

nucleotides (Table I). The nucleotide compositions of 4.5S RNA_I and 4.5S RNA_{III} are very similar to those reported earlier (5).

4.2S RNA - In addition, 4.2S RNA was visualized as a distinct spot between 4S RNA and 4.5S RNAs (Fig. 1). This RNA has a AMP+UMP+ψMP/GMP+CMP ratio of 0.81 (Table I) which is significantly greater than that of whole transfer RNA (11).

Separation of 5S RNA - The three 5S RNAs were separated earlier by chromatography on DEAE-Sephadex columns into 5S RNA_I, 5S RNA_{II} and 5S RNA_{III} (6). The two-dimensional procedure clearly separated these 5S RNA species from either nuclear 4-8S RNA (Fig. 1) or whole cell 4-8S RNA (Fig. 2). The nucleotide compositions of these three species (Table I) compare well to results published earlier (12).

Several minor spots labeled A, B and C (Fig. 1) were found that were not observed previously by one-dimensional slab gel electrophoresis. The nucleotide analyses of these species

TABLE I

NUCLEOTIDE COMPOSITIONS OF NUCLEAR RNA'S SEPARATED BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

RNA	AMP	UMP	GMP	CMP	ΨMP	NmpNp	$\frac{AMP+UMP+\Psi MP}{GMP+CMP}$
4.2S	18.0	23.9	27.9	26.7	2.4	1.3	0.81
$4.5S_I$	25.7	22.8	22.6	28.9	-	-	0.94
$4.5S_{II}$	20.6	23.7	30.0	25.7	-	-	0.80
$4.5S_{III}$	23.5	24.1	17.7	16.3	3.6	14.9	1.51
$5S_I$	18.5	23.7	30.2	27.8	-	-	0.73
$5S_{II}$	18.6	23.5	30.6	27.3	-	-	0.73
$5S_{III}$	21.8	32.0	21.1	19.7	1.6	3.8	1.36
A	23.6	32.0	22.0	22.6	-	-	1.25
B	22.1	29.2	24.8	23.9	-	-	1.05
C	20.3	26.2	27.7	25.8	-	-	0.87

The nucleotide compositions are percentages of the total ^{32}P in the RNA (8,9). The values are averages of two different experiments employing different RNA preparations.

indicate that they do not contain any ΨMP or 2'-O-methylated nucleotides (Table I).

DISCUSSION

The increased resolutions obtained by the two-dimensional acrylamide gel electrophoresis is apparent from the separation of the 4.5S and 5S RNAs, the appearance of the 4.2S RNA and minor spots A, B and C, and the separation observed in the transfer RNA region. The first dimension separates the RNA mainly on the basis of chain length. The second dimension of electrophoresis at pH 3.3 separates the RNA on the basis of chain length and base composition because the adenine and cytosine residues are protonated. The low pH and 6 M urea also disrupt base pairing

and aggregation that might affect the secondary structures of these molecules.

This method is very useful for the separation of individual RNAs from a mixture of RNAs as shown by its separation of the 4-8S RNAs or for isolating partial digestion products of an RNA for determining RNA sequence. It is possible that it will also be of value for separation of low molecular weight messenger RNA species and tRNA as shown by studies currently in progress in this laboratory.

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