THE SEPARATION OF α- AND β-RABBIT GLOBIN mRNA BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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1. Introduction

So far the separation of rabbit globin mRNA coding for α- and β-chains on the base of differences in molecular weight with common gel systems was unsuccessful. Therefore we made an attempt using polyacrylamide gel electrophoresis under denaturing conditions. This technique has shown to be most useful for the estimation of the molecular weight of mRNAs [1–4] and seems to be very suitable for the detection of precursors of mRNAs as by the use of formamide artificial aggregation of mRNA can be prevented.

We describe here the separation of rabbit globin mRNA, purified by zonal centrifugation and poly-dT cellulose chromatography, into two species which could be shown to direct the synthesis of α and β globin chains in a cell free system. The separation of α- and β-globin chains was achieved by polyacrylamide gel electrophoresis at pH 3.0 in the presence of urea.

2. Materials and methods

9S mRNA from rabbit reticulocytes was prepared as described [5], essentially according to the method of Lockard and Lingrel [6]. Purification on poly-dT cellulose (a generous gift of Dr U. Bertazzoni, Institut de Biologie Moléculaire, Paris) was performed as published elsewhere [7].

Polyacrylamide gel electrophoresis in the presence of formamide was performed according to Duesberg [8] with slight modifications. Formamide (analytical grade, Merck) was deionized by stirring with 5% (w/v) Zerolit (Permutit Comp) mixed bed ionic exchanger. After removal of the exchanger Na_2HPO_4 and NaH_2PO_4 were added to a concentration of 0.01 M each, resulting in a final pH of about 6.5.

8 × 0.5 cm 2.4% polyacrylamide gels were prepared according to Loening [9], except that H_2O was used instead of buffer. After polymerization the gels were removed from the tubes and soaked into 200 ml of phosphate buffered formamide. This solution was refreshed each 24 hr during 5 days. The gels could be stored at 4°C for several months in this solution. By this procedure gels were obtained devoided almost completely of any UV absorbing contamination. 24 hr before use the gels were suspended in freshly prepared formamide mixture. Then they were sucked back in the tubes and pre-electrophoresis was performed for 0.5 hr at 15 V/cm. As electrode buffer phosphate buffered formamide was used.

The RNA sample was dissolved in a minimal volume of water, whereafter 3 vol of phosphate buffered formamide solution containing 15% sucrose and 0.01% Bromophenol Blue were added. 4–10 μg of each RNA component was applied. Electrophoresis was performed at 15 V/cm until the Bromophenol Blue had migrated to the end of the gel.

After removal from the tubes the gels were scanned at 260 nm in a Gilford spectrophotometer, adapted with a gel scanner. After scanning the gels were spread on a glass plate which was wetted with some formamide in order to prevent deformation of the gel. The length was measured and the appropriate part was sliced into 2 mm slices with a razor blade. Extraction of the RNA from gel slices as indicated in the figures was performed by homogenizing the slices in 200 μl of H_2O containing 0.1 M LiCl. After leaving to stand this mixture for 2 hr at 4°C the gel material was removed by centrifugation. After reextraction of the gel

homogenate with 100 μl 0.1 M LiCl the supernatants were combined, 4 μg of carrier tRNA was added and the solution was extracted with one vol of chloroform in order to remove some white material which is insoluble in ethanol and presumably represents linear polyacrylamide polymers. The RNA in the water layer was precipitated overnight with 0.1 vol of 1 M NaAc, pH 5.2, and 2.5 vol of ethanol at −20°C.

Cell-free incubations in the wheat germ system, prepared according to Shih and Kaesberg [10] were performed as described [4].

Separation of α- and β-globin chains was obtained by gel electrophoresis at pH 3.0 in the presence of 6 M urea. Gels were prepared as described [11].

Electrophoresis was performed for 3–5 hr at 15 V/cm. After electrophoresis the gels were stained with amido Black and destained electrophoretically. Longitudinal slices were dried down on filter paper and autoradiographed with Kodak X-ray film [12].

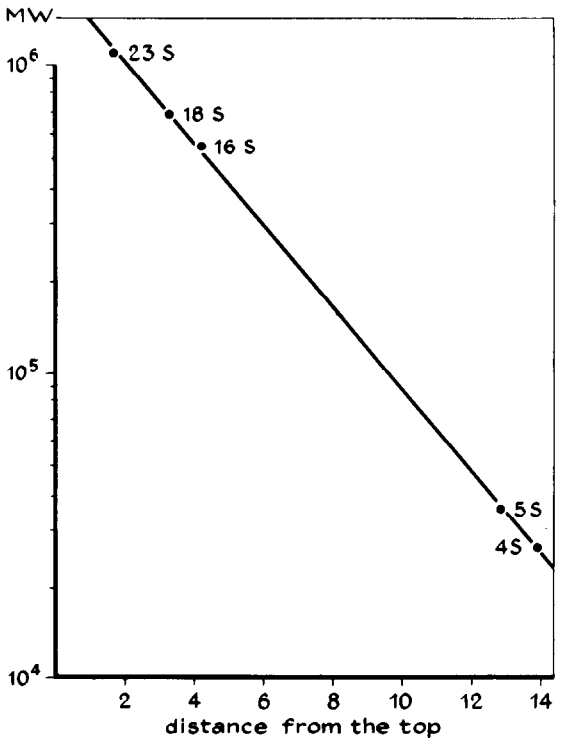

Fig. 1. Molecular weight-electrophoretic mobility relation for several RNA species in a 2.4% polyacrylamide gel with formamide as solvent. 4S, 5S and 18S RNA from reticulocyte ribosomes [18–21], 16S and 23S from *E. coli* [22].

Table 1
Stimulation of amino acid incorporation in the wheat germ cell-free system by RNA extracted from the gel slices 1–7 (fig. 2)

RNA fraction	CPM
1	1960
2	1130
3	1220
4	5620
5	3520
6	3960
7	2210
8	1420

Cell-free incubations in a wheat germ extract were performed in a volume of 25 μl as described in ref. [4]. One μl aliquots were assayed for precipitable radioactivity.

The relative amount of radioactivity in the α- and β-globin band was estimated by scanning the autoradiogram in a Gilford Spectrophotometer adapted with a film scanner.

3. Results and discussion

Formamide gels were calibrated with 18S, 5S and 4S RNA from reticulocytes and 23S and 16S RNA from *E. coli*. It appeared that by plotting the migration distance against the log molecular weight of the standard RNA's a straight line is obtained from which the molecular weight of the desired component can be calculated (fig. 1).

Electrophoresis of 9S mRNA from rabbit reticulocytes, purified by zonal centrifugation and poly-dT cellulose chromatography on these gels, resulted in the separation of this RNA into two species (fig. 2a). Extraction of the RNA from the gel slices in this region and translation of this RNA in a cell-free system derived from wheat germ revealed that both species were able to stimulate protein synthesis in this system (table 1). That the maximal stimulation does not coincide with fraction 5 (containing the highest amount of RNA) is probably caused by the fact that with this fraction the amino acid incorporation is reduced by a too high concentration of mRNA. SDS gel electrophoresis followed by autoradiography (not shown) demonstrated that both species directed the synthesis of polypeptides comigrating with carrier

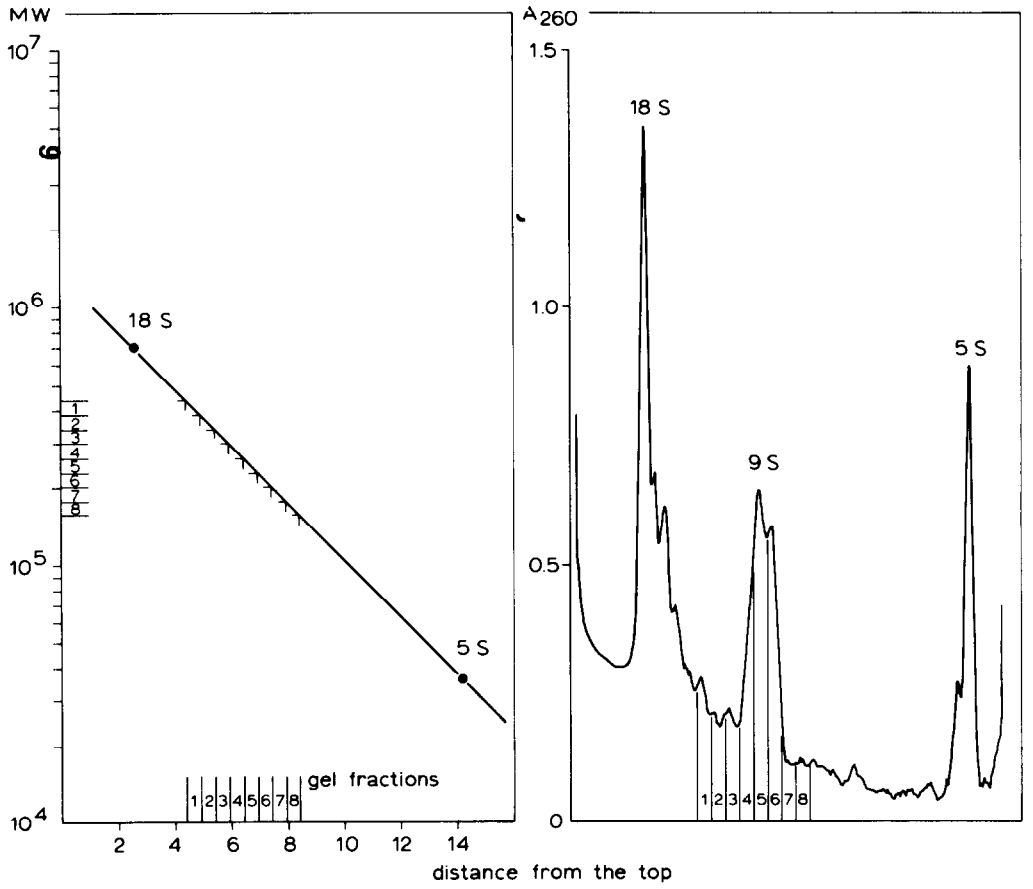

Fig. 2. Separation of rabbit globin mRNA by polyacrylamide gel electrophoresis in the presence of formamide. 5S and 18S RNA from reticulocyte ribosomes were added as molecular weight markers. Gel electrophoresis was performed as described in the Materials and methods section.

globin. A slight amount of globin was also synthesized by a fraction located near the 18S ribosomal RNA, which was added together with 5S as a molecular weight marker. Since the 18S marker was derived from rabbit reticulocyte polyribosomes and aggregation of mRNA is unlikely in gels containing formamide, this species might represent a precursor of 9S mRNA which contaminated the 18S preparation.

This putative precursor which should have a mol. wt. of about 400 000 and which could be the same species described by Maroun et al. [13] was not further identified.

Identification of the polypeptides synthesized under the direction of the RNA's from the 9S region with the aid of acidic urea gels [11] revealed that the slowest moving component directed the synthesis of predominantly β-chains whereas the fast moving component coded for the α-chain of rabbit globin (fig. 3).

Although it cannot be stated definitively that the two species seen in the OD scanning represent exclusively α- and β-globin mRNA, the results of the translation experiments suggest that these two peaks represent indeed β- and α-globin mRNA. On the other hand the relatively high amount of β-messenger is in contradiction with the expected ratio based on translational studies [14]. However, the change ratio can be explained by assuming that during isolation of reticulocyte polyribosomes an enrichment of β-polysomes was obtained as the centrifugation times for sedimenting the polysomes were limited. Further-

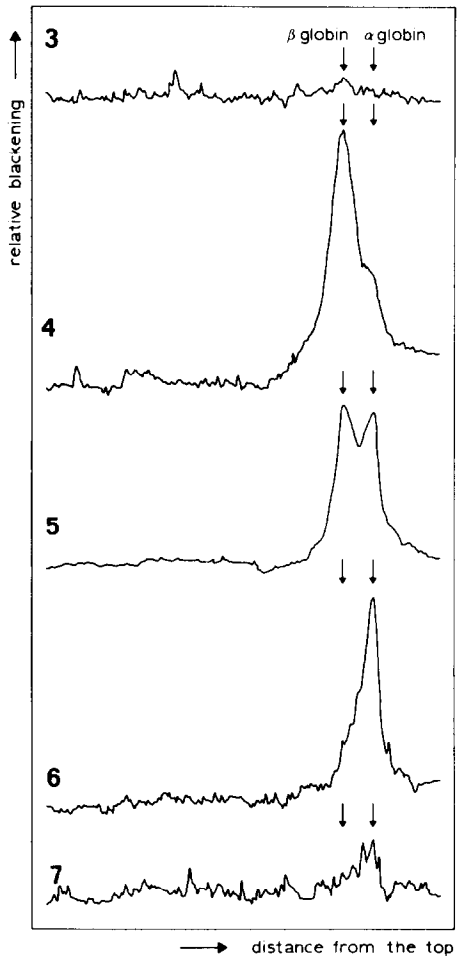

Fig. 3. Analysis of the products synthesized under the direction of RNA from fraction 3–7 (see fig. 2) on acidic urea gels. Scannings of the autoradiograms are shown. The position of the β- and α-chain is marked in the figure. Incubation and analysis were performed as described in the Materials and methods section.

more one cannot exclude that the poly-dT cellulose chromatography may have resulted in an enrichment of β-mRNA, caused by the presence of a longer poly A track in this mRNA, although experiments with mouse globin mRNA do not support this assumption [15]. Assuming that the maximal optical density coincides with the position of the messengers we estimated the mol. wt. for α- and β-mRNA to be 210 000 and 240 000 respectively (fig. 2b) which is close to the values obtained for globin mRNA by other authors [1,16,17].

This difference in mol. wt. between α- and β-globin mRNA is much greater than 5000 as could be expected on base of the difference in amino acid composition [23]. Therefore the bulk of extra bases in the β-mRNA has to be present in extracistronic regions: in the poly A track (which would fit very well with the observed enrichment of β-mRNA, after the purification procedure used), in other untranslated regions, or in both. Which of these alternatives represent the actual situration awaits isolation of α- and β-mRNA on a preparative scale and the determination of the length of their individual poly A track.

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