THE MOLECULAR WEIGHT OF THE 14S CALF LENS MESSENGER RNA

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SUMMARY

The molecular weight of the 14S messenger fraction from calf lens which codes for the A_2 chain of α-crystallin (1) was determined by polyacrylamide gel electrophoresis in the presence of formamide.
After electrophoresis the localization of the messenger was established by extracting successive gel slices and testing the coding ability of the extracted RNA in the ascites or wheat germ cell-free system. It appeared that the maximal absorbance of the crude 14S messenger did not coincide with the biological active fractions. Whereas the maximal absorbance was found in a molecular weight range around 380,000 the messenger activity was located at 510,000 dalton. After purification of the 14S messenger on poly(dT)-cellulose the mRNA migrated as a single sharp band. In the latter case the optical density coincided exactly with the biological activity.

INTRODUCTION

Molecular weight determinations of mRNA's by polyacrylamide gel electrophoresis under denaturing conditions have been described for globin mRNA's (2, 3) and immunoglobin light chains (4). The molecular weight determination by polyacrylamide gel electrophoresis in the presence of formamide (5) seems to be the most sophisticated procedure available at the moment. However, since almost all mRNA preparations are more or less contaminated with other RNA species, one encounters the difficulty to decide which component in the gel represents the true messenger. One can overcome this problem by identifying the components in the gel by laborious sequence studies (4) or by the rather simple procedure described in the present paper.

MATERIALS AND METHODS

14S mRNA from calf lens was prepared as described earlier (6). Polyacrylamide

gel electrophoresis in the presence of formamide was performed according to Duesberg (7) 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 x 0.5 cm 2.4% polyacrylamide gels were prepared according to Loening (8), except that H_2O was used instead of buffer. After polymerization the gels were removed from the tubes and put into 200 ml of phosphate buffered formamide. This solution was refreshed each 24 h during 5 days. The gels could be stored at 4^oC for several months in this solution. By this procedure gels were obtained devoid almost completely of any UV absorbing contamination.

24 h 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 ½ h 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 volumes of phosphate buffered formamide solution containing 15% sucrose and 0.01% Bromophenol blue were added. 4 - 10 µg of each RNA component was applied. Electroporesis 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 h at 4^o the gel material was removed by centrifugation. After reextraction of the gel 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 volume of chloroform. The RNA in the water

layer was precipitated overnight with 1/10 volume of 1 M NaAc, pH 5.2, and 2½ volume of ethanol at -20°.

Cell-free incubations in the ascites system were performed as described earlier (9). For the wheat germ system the protein synthesis reaction was performed in a final volume of 25 µl containing the following components: 10 µl wheat germ extract prepared according to Shih and Kaësberg (10), except that the preincubation step was omitted, 12 mM HEPES, pH 7.5, 60 mM KCl, 3 mM MgAc, 1 mM ATP, 0.2 mM GTP, 8 mM phosphocreatine, 25 µg kinase, cold amino acids minus methionine as described by Palmiter (11), 1 µCi [^{35}S]-methionine (180 Ci/mmol) and 0.5 µg of mRNA. Incubation was carried out for 2 h at 30°C. Synthesized products were analyzed by SDS gel electrophoresis followed by autoradiography of the longitudinal gel slices (6).

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). In gels used for the determination of the molecular weight of the 14S messenger only 18S, 5S and 4S were co-electrophoresed in order to prevent overlapping with the 16S RNA from E. coli.

A typical optical density profile of a 14S sample, purified by zonal centrifugation, together with 18S, 5S and 4S RNA is shown in fig. 2. The maximal absorbance of the 14S material is found at a position corresponding with a molecular weight of 380,000, close to the value of 360,000, reported earlier by one of us for 14S RNA (12) using the method of Boedker (13). However, when the coding ability of the RNA from successive gel slices was tested 14S messenger activity was only found in fractions 2 and 3 corresponding to a molecular weight of about 500,000 dalton (fig. 3). In order to verify whether

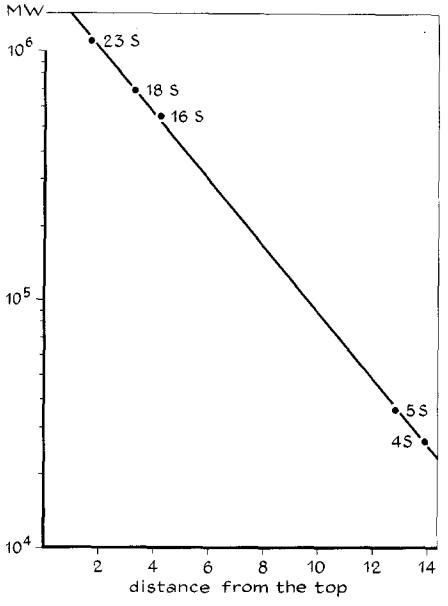

Fig. 1. Molecular weight - electrophoretic mobility relation for several RNA species in a 2.4% polyacrylamide gel with formamide as solvent. The RNA's used as references are:
4S, 5S and 18S RNA from reticulocyte ribosomes (21, 22, 23, 24),
16S and 23S RNA from E. coli (25).

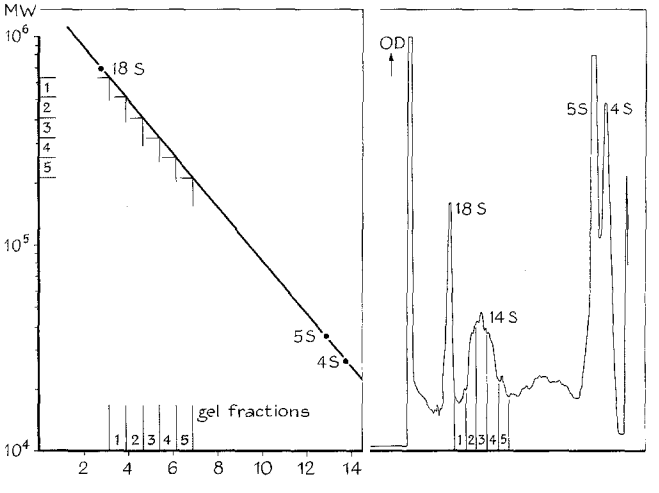

Fig. 2. Molecular weight determination of a 14S calf lens mRNA preparation after zonal centrifugation but before poly(dT)-cellulose chromatography.
Gel electrophoresis was performed as described in the method section.

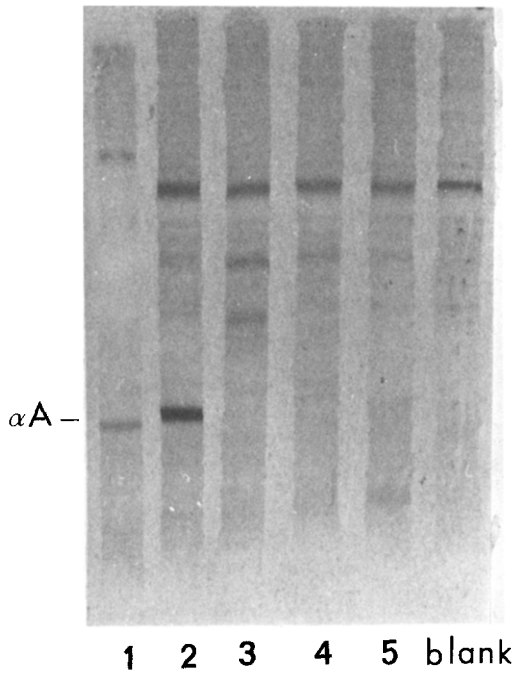

Fig. 3. Analysis of the products synthesized under the direction of RNA fraction 1 - 5 (see Fig. 2) in the Ehrlich ascites cell-free system. Incubation and analysis were performed as described in the method section.

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further purification of the 14S fraction resulted in a shift of absorbance from the 380,000 to the 500,000 position the 14S RNA was absorbed to poly(dT)-cellulose (14) and the messenger eluted at 50°C. This material migrated as a sharp band in formamide gels at the 500,000 MW position (fig. 4). In this case messenger activity coincided completely with the optical density of the 500,000 component (fig. 5). In order to establish the molecular weight more accurately the molecular weight determination was repeated several times with different 14S preparations. This yielded a molecular weight of 510,000 $\pm$ 40,000 for the biological active 14S messenger. Moreover, no indication of heterogeneity was found by this technique. The observed molecular weight for αA_2 crystallin messenger seems to be extremely high, since the messenger contains 1460 bases of which only 519 are required for the αA_2 cistron (10). Subtracting the 200 adenine residues present (9), there are about 720 bases in excess.

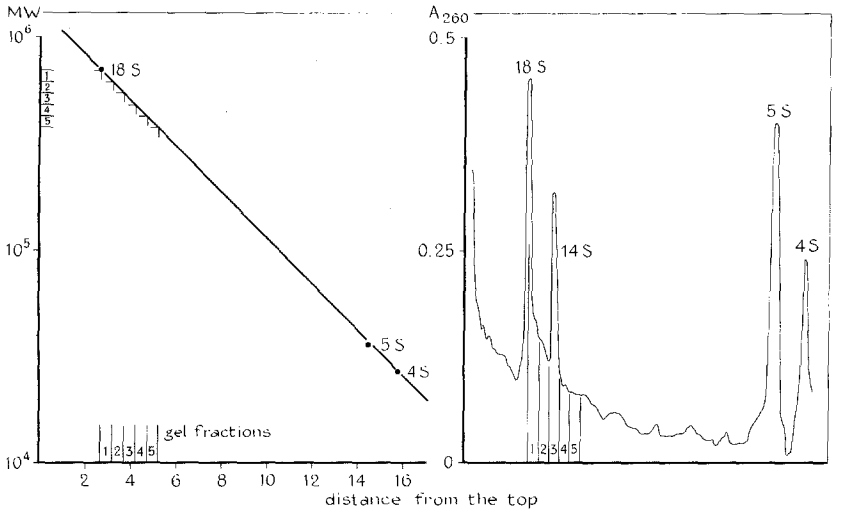

Fig. 4. Molecular weight determination of 14S calf lens mRNA after purification on poly(dT)-cellulose (14).
Electrophoresis and extraction was performed as described in the method section.

It is surprising that the other major polypeptide chain of α crystallin, αB_2, which has 55% homology with αA_2 (15, 16) and which exhibits a similar size (17) is encoded for by an mRNA of about 250.000 MW (12). The homology suggests strongly that both chains are derived from a common ancestor gene. Since both chains have the same N-terminal sequence N acetyl-Met-Asp-Ile-Ala (15, 16) the initiation site has been conserved. As both messengers contain poly A the sequence adjacent to the poly A may well be the last transcription product of the DNA dependent RNA polymerase and could represent part of a recognition site for the poly A adding enzymes (18, 19). Assuming that also this sequence is conserved in both messengers, which seems quite reasonable for such an important signal, then the large excess of bases in the αA_2 messenger should be located at the 5' end. Since it has been shown that the N-terminal Met is derived from the initiator tRNA (1), this allows the prediction that the synthesis of a precursor molecule (which should, as a consequence, have its extension at the C-terminal end) cannot be the explanation for the extremely large size of the message. Unless this excess is due to the occurrence of a

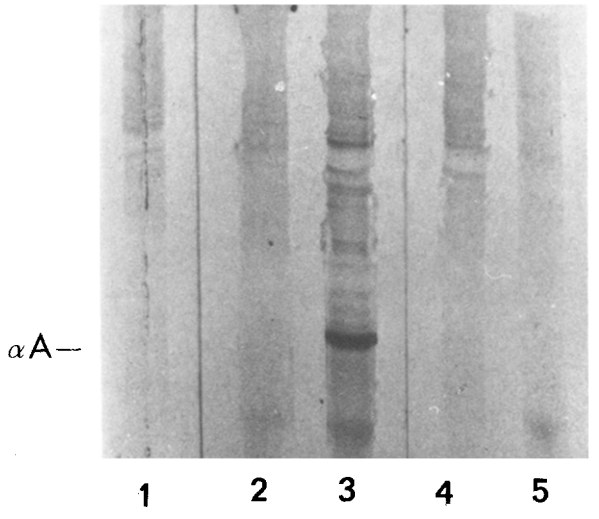

Fig. 5. Analysis of the products synthesized under the direction of RNA fractions 1 - 5 (see Fig. 4) in the wheat germ cell-free system.

second cistron, whose expression could not be traced hitherto, the most likely explanation for our observation would be that besides the recognition region, located probably near the initiation site (as should be the case by the closely related αB_2 messenger) a large nucleotide sequence is present at the 5' end with no direct function on the translational level. However, one may speculate that this region plays a role in the suggested higher stability of the αA_2 messenger as compared to the αB_2 mRNA (20). The difference in length between these two related species could most probably have been arisen from a mutation by which the processing of their messenger precursors is altered.

Our results show that a combination of polyacrylamide gel electrophoresis under denaturing conditions together with a test for biological activity is probably the most accurate approach available at present for the determination of the molecular weight of messenger RNA's, especially of partly purified mRNA species.

Further we want to emphasize that the wheat germ system is very suitable for testing lens mRNA's which are translated in this system as efficiently as globin mRNA.

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