THE MOLECULAR WEIGHT OF RABBIT GLOBIN MESSENGER RNA'S

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1. Introduction

Previous molecular weight measurements of rabbit globin messenger RNA (mRNA) by polyacrylamide gel electrophoresis [1, 2] have been performed in aqueous systems in which the secondary structure of the molecules is conserved. In this study formamide was used to destroy all secondary structure [3] of the RNA (both mRNA and standards) so that conformational changes do not affect the electrophoretic mobility and therefore the distance migrated in a gel by an RNA species is proportional to the log. molecular weight only.

2. Methods

2.1. *Preparation of RNA*

A lysate was made of the red blood cells from anaemic rabbits [4] and polysomes obtained by centrifuging this for 1 hr at 30,000 rpm (105,000 *g*) [5]. mRNP (i.e. messenger-ribonucleic protein) was released from the polysomes by suspension in 10 mM Tris, 33 mM EDTA pH 7.4 and isolated by centrifugation in a zonal rotor [6] (see fig. 1 for details). The mRNP was precipitated from the gradient fractions by making them 0.1% SDS, 0.2 M NaCl then adding 2 vol of ethanol. The precipitate was kept at −20° for several hours and then the proteins removed by the phenol/SDS method [7]. After reprecipitating three times the final precipitate was washed twice with 70% ethanol and dried in vacuo.

The RNA's used as standards had also been treated to ensure as complete removal of proteins as possible [8]. These were μ2, 23 S, 16 S, 18 S, 5 S and 4 S.

2.2. *Formamide gel electrophoresis*

The method used was based on that described by Staynov et al. [9]. The following modifications were suggested by J. Pinder (personal communication): the

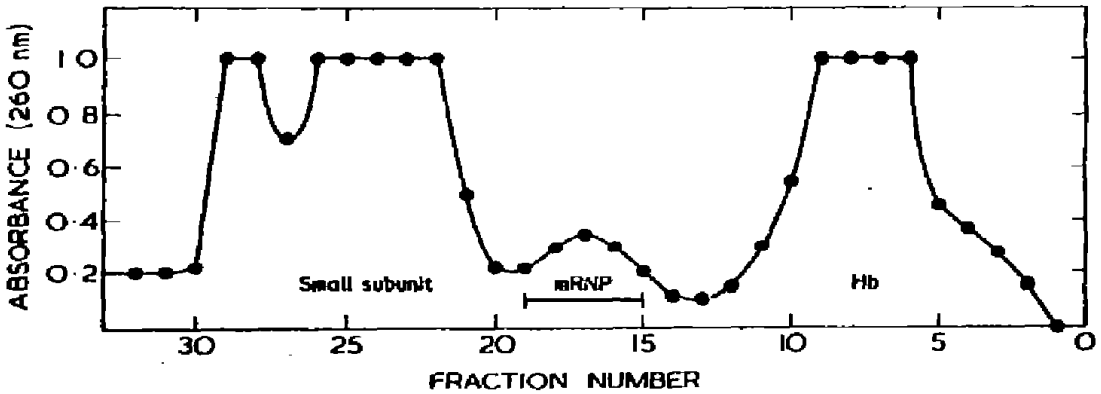

Fig. 1. Polysomes suspended in 20 ml of 10 mM Tris–33 mM EDTA, pH 7.4, were layered onto a sucrose gradient, 10% sucrose–10 mM Tris, pH 7.4, to 40% sucrose–10 mM Tris, pH 7.4, then an overlay of 30 ml 10 mM Tris was added. Centrifugation was for 16 hr at 48,000 rpm and 4° in the Spinco BXIV. Abbreviations: Hb = haemoglobin, mRNP = messenger ribonucleoprotein.

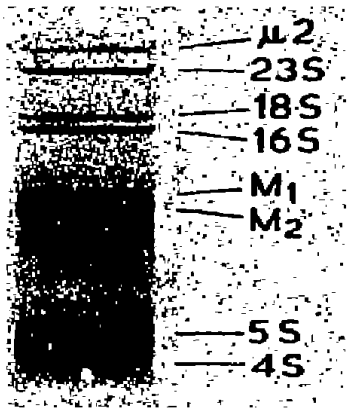

Fig. 2. Disc electrophoresis of μ2 RNA, 24 S, 18 S, mRNA, 5 S and 4 S.

use of amberlite instead of Dowex to deionise the formamide; buffering the formamide at pH 9 with 0.02 M diethyl barbituric acid; the use of disc gels rather than flat bed gels.

3.5% Polyacrylamide gels (of which 15% was bisacrylamide) were used to separate a mixture of mRNA and RNA's of known molecular weight. The mixture was dissolved in deionised, buffered formamide plus 6% sucrose and 0.05% bromophenol blue and layered on top of the gel under deionised buffered formamide. The electrolyte – 0.02 M NaCl was circulated between the compartments of the electrophoresis apparatus during the run. Electrophoresis was 100 V, 10 mA for 4 gels (7 mm in diameter). After 3 hr the gels were stained with 0.1% Pyronin Y, 0.5% acetic acid, 1 mM citric acid, and destained with 0.5% acetic acid (fig. 2).

3. Results

Stained gels were scanned in a Gilford scanner so that the distance migrated by each RNA from the origin could be accurately measured. By plotting the distance migrated against log. molecular weight of the standard RNA's a straight line is obtained from which the molecular weight can be calculated (fig. 3). The distance migrated by the same RNA species in different gels is not always reproducible for reasons not fully understood. For this reason the curves in fig. 3 have different slopes, and internal markers must be used. As can be seen from fig. 2 the mRNA (which runs as one band in aqueous gels [1, 2]) splits into two bands. These are labelled M_1 and M_2 (M_1 is the larger). M_1 has a molecular weight of 227,000 (standard deviation 3.1%) and M_2 of 202,000 (standard deviation 2.7%). See table 1. It is assumed that these bands represent α and β mRNA since the RNA used was made from 14 S mRNP which has been shown to be a purer source of mRNA than 9 S RNA [16]. Kazazian

Table 1
The molecular weight of rabbit haemoglobin messenger RNA's.

	Measured MW (8 determinations)	Expected MW*	Excess MW (measured–expected)	Excess expressed in no. of nucleotides
M_1 = β mRNA	227,000 SD = 3.1%	140,160	86,840 SD = 8.1%	271 SD = 8.1%
M_2 = α mRNA	202,000 SD = 2.7%	135,360	66,640 SD = 8.3%	280 SD = 8.3%

* Expected MW calculated using number of amino acids in chain [18], average nucleotide MW of 320 and coding ratio of 3 amino acids/nucleotide [19].

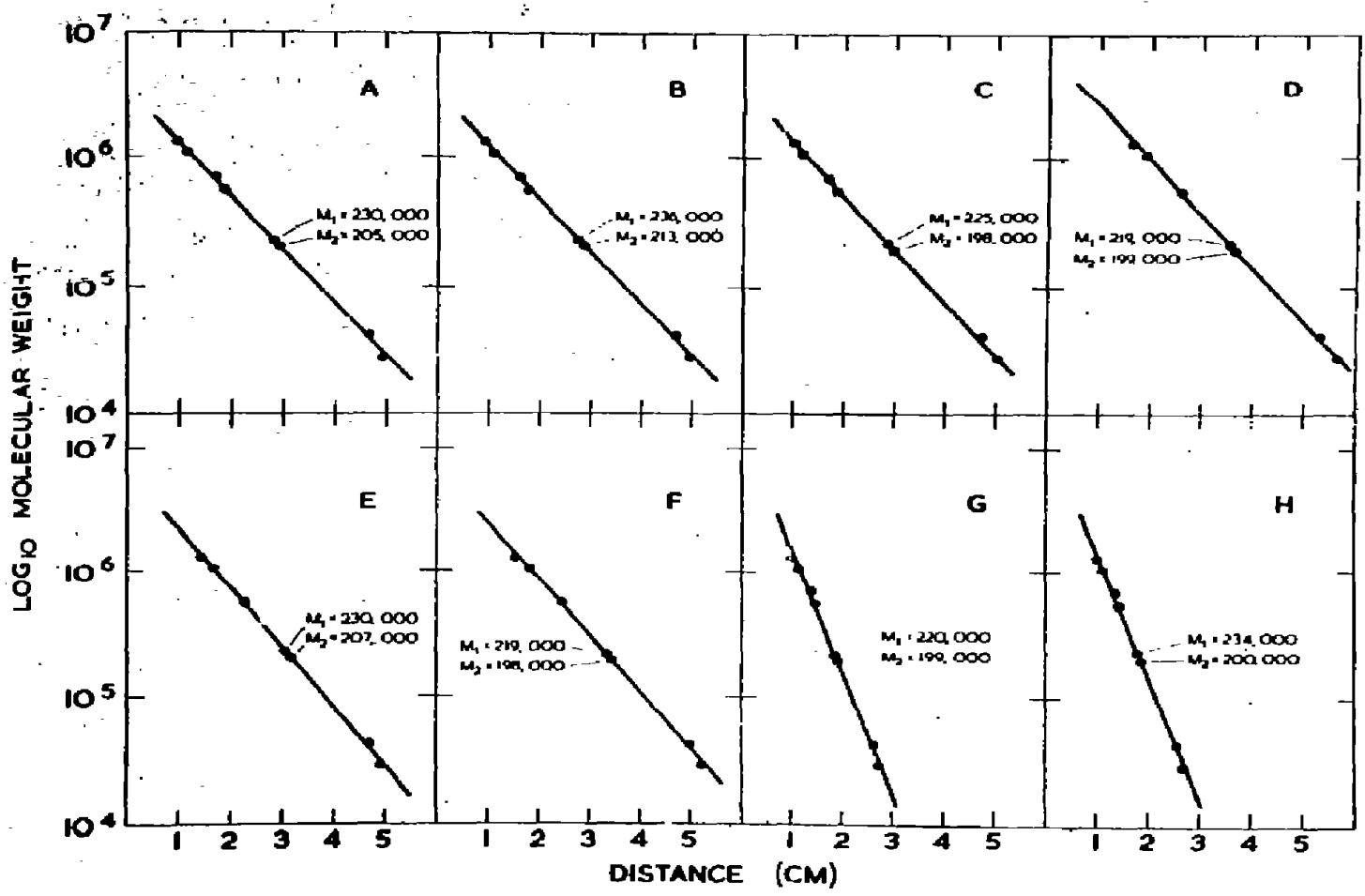

Fig. 3. Data used for the calculation of the molecular weight of M_1 and M_2. The standards used were (in descending order) $\mu 2$ phage (essentially the same as R17) [10], 23 S from *E. coli* [11], 18 S from rabbit reticulocytes [12], 16 S from *E. coli* [13], 5 S [14] and 4 S [15] from rabbit reticulocytes.

has shown that mRNA for β globin is larger than mRNA for α globin [17] and so M_1 is equated with β mRNA.

4. Discussion

Other studies of rabbit haemoglobin mRNA on polyacrylamide gels have concentrated either on measuring the molecular weight (for which one value is obtained), at an acrylamide concentration which allows internal standards to be included [1, 2], or demonstrating the heterogeneity of the RNA by using a higher gel concentration [17]. The present study combines these two since in formamide two mRNA's separate at a gel concentration suitable for use with a wide range of marker RNA's of known molecular weight. In addition, this method should provide increased accuracy in molecular weight determination due to the removal of secondary structure in the RNA. The results obtained in these experiments are at the upper end of the range of molecular weights already published. Chantrenne et al. [20] calculated a value of 150,000 from measurements of the sedimentation coefficient. Using polyacrylamide gels – a more sensitive method, Gaskill and Kabat [1] obtained a molecular weight of 220,000, Labrie [2] 190,000 and Kazazian 180,000 (personal communication).

There is a difference of about 3% in the number of amino acids in the two globin chains (β has 146 and

α 141 [18]). It might be supposed that the mRNA's would reflect this difference. However, Kazazian reports a 5% difference and we find it is about 10%. This corresponds to an extra 60 nucleotides whereas the expected difference is 15. Lim and Canellakis [21] have reported that a region of rabbit globin mRNA between 50 and 70 nucleotides long is over 70% adenine. This 'poly-A' rich region is thought unlikely to be informational [22]. Their experiments do not distinguish between α and β mRNA although they observe that there is only about half as much poly-A in mRNA obtained from lighter polysomes. It is known that in lighter polysomes there is more α mRNA than β mRNA [23]. Therefore it is possible that α mRNA contains appreciably less poly-A and that β mRNA is larger because it contains this adenine rich sequence.

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