# MOLECULAR WEIGHT OF THYROGLOBULIN 33 S MESSENGER RNA AS DETERMINED BY POLYACRYLAMIDE GEL ELECTROPHORESIS IN THE PRESENCE OF FORMAMIDE

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## 1. Introduction

The precursor of thyroid hormones, thyroglobulin is one of the largest known globular proteins (mol. wt 660 000, sedimentation coefficient 19 S) [1]. Recently, a 33 S poly(A)-rich messenger RNA has been isolated from membrane-bound thyroid polysomes and shown to code for the two 300 000 mol. wt protomers of thyroglobulin [2,3]. Since all previous models proposed for thyroglobulin quaternary structure involved a larger number of smaller subunits [1,4–8] it was of interest to demonstrate that the 33 S mRNA could indeed cope with the amount of genetic information required for the synthesis of a 300 000 mol. wt polypeptide chain. Therefore, it was the aim of this study to estimate the molecular weight of thyroglobulin mRNA. Polyacrylamide gel electrophoresis in the presence of formamide was used because it has been shown to minimize the influence of secondary structure on molecular weight determinations [9,10]. An apparent mol. wt of $2.8 \times 10^6$ has been obtained. Also a new procedure of 33 S mRNA purification is presented resulting in cleaner preparations.

## 2. Materials and methods

### 2.1. *Preparation of thyroglobulin mRNA*

Beef thyroid glands were obtained from a local slaughterhouse within a few minutes after animal death. They were immediately trimmed free of connective tissue and fat, cut into small fragments ($\sim$ 1 $cm^3$), frozen in liquid nitrogen and stored thereafter at –60°C. Hundred gram portions of tissue were powdered under liquid nitrogen, and homogenized in 4 vol. 200 mM Tris–HCl, pH 8.5, 50 mM KCl, 25 mM $MgCl_2$ 3 mg/ml yeast RNA (Koch Light), 200 mM sucrose by five strokes of a Teflon glass homogenizer [11]. The homogenate was spun at 27 000 × *g* for 10 min (SS 34 Rotor-Sorvall).

The resulting pellet containing the majority of the thyroglobulin synthesizing polysomes [11] was suspended in 40 ml homogenization buffer containing 2% Triton X-100 and incubated for 10 min at 0°C. The mixture was then centrifuged for 10 min at 27 000 × *g* and the resulting supernatant saved. Ten ml samples of this supernatant were layered onto discontinuous sucrose gradients consisting of 3 ml 70% sucrose, 10 ml 40% sucrose and 13 ml 20% sucrose prepared in 50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 0.5 mg/ml heparin. The gradients were centrifuged for 110 min at 25 000 rev/min (SW 27 Rotor Beckman) and the material at the 40%/70% sucrose interface together with the 70% sucrose layer and the pellet were collected. This fraction is enriched in the large membrane-bound polysomes which have been shown to the responsible for thyroglobulin synthesis [12]. The mixture was brought to 10 mM EDTA and 1% sodium dodecyl sulphate, then extracted with phenol/chloroform as described previously [2]. Poly(A)-rich RNA was prepared as already described [2], except that two rounds of poly(U)–Sepharose were performed.

*Abbreviations*: mRNA, messenger RNA; rRNA, ribosomal RNA; poly(A), polyadenylic acid; poly(U), polyuridilic acid

2.2. *Electrophoretic procedure*

Polyacrylamide gel electrophoresis in the presence of formamide was performed according to Duesberg and Vogt [13] with the following modifications. Gels (2.7% or 3%) were prepared in 10% glycerol and overlaid with 100 μl of phosphate buffered formamide containing the catalysts to improve the quality of the interface. The water content of the gels was only that brought by the hydrated phosphate salts and by the ammonium persulfate solution (250 μl/20 ml). The gels were subjected to electrophoresis (1.5 mA/tube) for 6 h (2.7% gels) or for 18 h (3% gels). They were stained with 'Stains All' as described [14].

## 3. Results and discussion

Figure 1 illustrates the sedimentation pattern of poly(A)-rich RNA extracted from the large membrane-

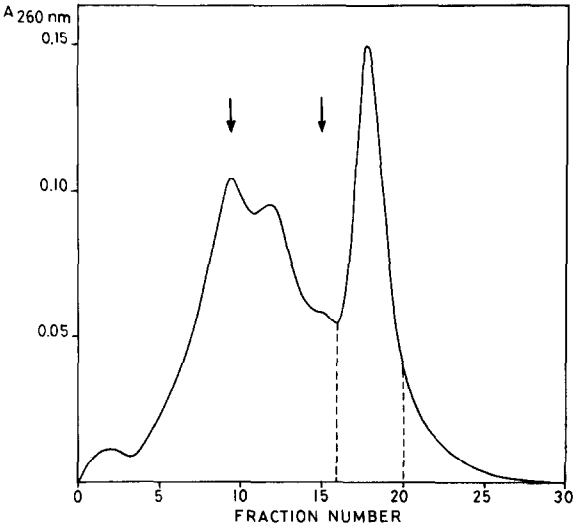


Fig.1. Sedimentation pattern of poly(A)-rich RNA from large membrane-bound polysomes. Poly(A)-rich RNA was purified by two rounds of poly(U)–Sepharose from the total RNA extracted from the large membrane-bound polysomes (see methods). Following ethanol precipitation, the RNA was dissolved in 200 μl 10 mM Tris–HCl pH 7.5, 10 mM NaCl, 1 mM EDTA, briefly heated to 80°C and layered onto 5–30% linear sucrose gradients prepared in the same buffer. Centrifugation was for 150 min at 60 000 rev/min. The $A_{260}$ nm pattern was recorded as described [12]. RNA from the 33 S peak was precipitated with ethanol and used for molecular weight determinations (figs 2,3). The arrows point to positions reached by 18 S and 28 S rRNA centrifuged simultaneously in a separate gradient.

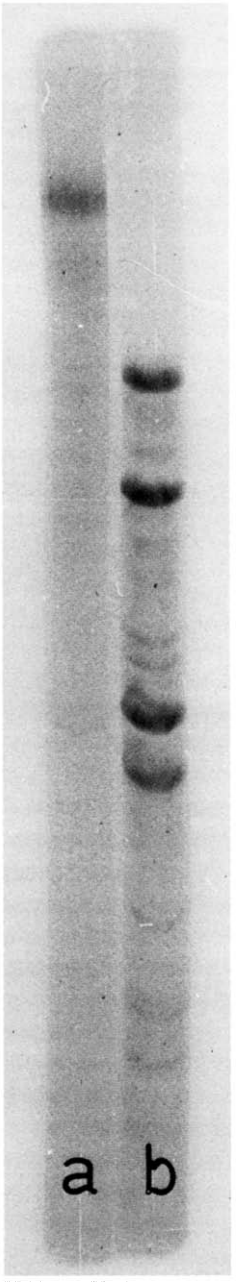


Fig.2. Polyacrylamide gel electrophoresis of thyroglobulin mRNA in the presence of formamide: (a) 3 μg of thyroglobulin mRNA; (b) 5 μg of thyroid rRNA, plus 5 μg total RNA from *Bacillus stearothermophilus*.

bound polysomes prepared by the discontinuous sucrose gradient method (see preceding section). As compared to results published previously [2,3], a larger proportion of intact 33 S mRNA could be recovered since it represented the largest peak observed throughout the gradient. The RNA in the 33 S peak was recovered by ethanol precipitation and used as such for analysis by polyacrylamide gel electrophoresis in formamide. Routinely, about 10 μg 33 S RNA were obtained from 100 g beef thyroid tissue.

One to five micrograms of thyroglobulin mRNA were run either on 2.7% or 3% gels, together with rRNA markers (18 S, 28 S from beef thyroid; total RNA from *Bacillus stearothermophilus*) in the same gel or in gels run in parallel. Figure 2 illustrates the results obtained in 3% gels. The 33 S RNA exhibited a single band migrating much more slowly than 28 S rRNA. Estimations of the molecular weight of thyroglobulin mRNA in 3% and 2.7% gels could only be obtained by extrapolation of a standard curve plotted from the migration of the RNA markers (fig.3a,b). Unfortunately, due to the large size of thyroglobulin mRNA we could not get marker RNAs migrating more slowly than the 33 S RNA. The average molecular weight obtained from six independent measurements was $2.8 \times 10^6$ (range: $2.6–3.0 \times 10^6$). No systematic differences were observed between the results obtained in 3% and 2.7% gels. Heating of the RNA for 5 min to 60°C prior to electrophoresis did not change the apparent molecular weight.

The minimal molecular weight of a mRNA coding for a 300 000 mol. wt polypeptide would be around $2.5 \times 10^6$. Some uncertainties persist in the molecular weight determination of nucleic acids by polyacrylamide gel electrophoresis, even in the presence of formamide [13,15]. Nevertheless, it seems legitimate to conclude from the above data that the 33 S thyroglobulin mRNA is large enough to contain the 7800 bases required for the synthesis of a 300 000 mol. wt polypeptide together with the 3′-poly(A) sequence and other non-translated sequences.

Thyroglobulin mRNA is the largest non-viral mRNA described today in the mammal.

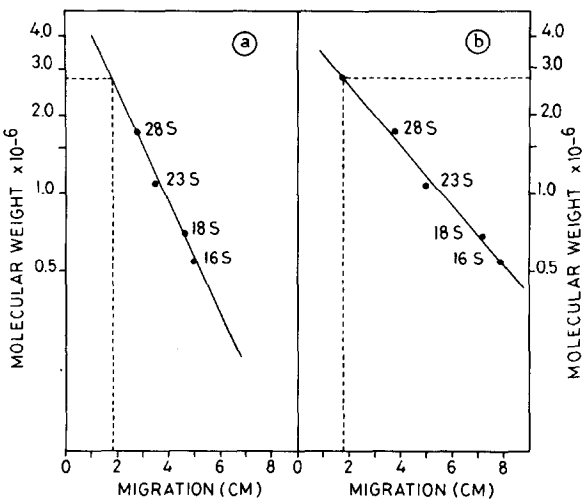


Fig.3. Polyacrylamide gel electrophoresis of thyroglobulin mRNA in the presence of formamide. The electrophoretic migration of thyroglobulin mRNA in 2.7% (a) or 3% (b) acrylamide gels has been compared to the migration of beef ribosomal RNA and total RNA from *Bacillus stearothermophilus*. The following molecular weights have been assigned to eukaryotic and procaryotic rRNA according to refs [16] and [17]. 28 S, $1.75 \times 10^6$; 23 S, $1.1 \times 10^6$; 18 S, $0.68 \times 10^6$; 16 S, $0.55 \times 10^6$.

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## References

[1] Edelhoch, H. (1965) Rec. Progr. Horm. Res. 21, 1–31.
[2] Vassart, G., Brocas, H., Lecocq, R. E. and Dumont, J. E. (1975) Eur. J. Biochem. 55, 15–22.
[3] Vassart, G., Refetoff, S., Brocas, H., Dinsart, C. and Dumont, J. E. (1975) Proc. Natl. Acad. Sci. USA 72, 3839–3843.

[4] Charlwood, P. A., Pitt-Rivers, R. and Schwartz, H. L. (1970) Biochem. J. 116, 769–772.
[5] Pierce, J. G., Rawitch, A. R., Brown, D. M. and Stanley, P. G. (1965) Biochim. Biophys. Acta 111, 247–256.
[6] Vecchio, G., Carlomagno, M. S. and Consiglio, E. (1971) J. Biol. Chem. 246, 6676–6682.
[7] Lissitzky, S., Rolland, M., Reynaud, J., Savary, J. and Lasry, S. (1968) Eur. J. Biochem. 4, 464–471.
[8] Spiro, M. J. (1973) J. Biol. Chem. 248, 4446–4460.
[9] Staynov, D. Z., Pinder, J. C. and Gratzer, W. B. (1972) Nature New Biol. 235, 108–110.
[10] Spohr, G., Mirault, M. E., Imaizumi, T. and Scherrer, K. (1976) Eur. J. Biochem. 62, 313–322.
[11] Davies, E., Dumont, J. E. and Vassart, G. (1977) Analyt. Biochem. in press.
[12] Vassart, G. and Dumont, J. E. (1973) Eur. J. Biochem. 32, 322–330.
[13] Duesberg, P. H. and Vogt, P. K. (1973) J. Virol. 12, 594–599.
[14] Dahlberg, A. E., Dingman, C. W. and Peacock, A. C. (1969) J. Mol. Biol. 41, 139–147.
[15] Woo, S. L., Rosen, J. M., Liarakos, C. D., Choi, Y. C., Busch, H., Means, A. R., O'Malley, B. W. and Robberson, D. L. (1975) J. Biol. Chem. 250, 7027–7039.
[16] Wellauer, P. K., David, I. B., Kelley, D. E. and Perry, R. P. (1974) J. Mol. Biol. 89, 397–407.
[17] Attardi, G. and Amaldi, F. (1970) Ann. Rev. Biochem. 39, 183–226.