Structure and biological activity of polysomes stained with Coomassie blue

M.R.V. Murthy, N. Hanna, A.D. Bharucha, R. Charbonneau, J.-L. Viallard+ and B. Dastugue+

Department of Biochemistry, Faculty of Medicine, Laval University, Québec G1K 7P4, Canada and +*Laboratoire de Biochimie Médicale, Faculté de Médecine, Université de Clermont-Ferrand 1, Clermont-Ferrand, France*

Received 22 August 1985

Polysomes prestained with Coomassie blue were fractionated on sucrose density gradients giving rise to visible bands corresponding to different size classes of aggregates. Coomassie blue staining enhanced the capacities of brain and liver polysomes to synthesize proteins in vitro, including the synthesis of neuron-specific enolase. This positive action of the dye was restricted to polysomes and was not manifested when mRNAs isolated from prestained polysomes were tested in in vitro translation or reverse transcription, indicating that the action of the dye consists in stabilization of polysomal structure.

Coomassie blue staining *Polysome activity* *Polysome structure* *Neuron-specific enolase synthesis* *Brain mRNA translation*

1. INTRODUCTION

Coomassie blue has been used as a stain for the detection and analysis of proteins in biological fluids [1], on paper and cellulose acetate strips [2] and on agar, starch gel [2] and polyacrylamide gels following electrophoresis [3–5]. The dye-protein complex possesses an intense blue color with an absorption maximum around 549 nm. When used for the analysis of proteins on electrophoretic strips or gels, the protein bands are generally fixed in acid and the staining is carried out at low pH in media to which ethanol or methanol is sometimes added. However, Coomassie blue is shown to bind to protein even in mildly alkaline aqueous buffers with a high molar ratio of dye to protein in the complex [6].

In the course of our work on the regulation of protein biosynthesis at the cytoplasmic level, we have been interested in the binding of various chemicals to the RNA or protein constituents of the translational apparatus and the effects of such binding on the biological activities of these macromolecules [7]. We report here that polysomes stained under mild conditions with Coomassie blue give rise to colored complexes which can be fractionated by centrifugation on sucrose density gradients. Visible blue bands corresponding to different size classes of polysomes are obtained. Staining of brain or liver polysomes with Coomassie blue enhances their activity in protein synthesis, including the synthesis of neuron-specific enolase in the case of brain polysomes. The positive effects of Coomassie blue in translation are restricted to polysomes and are abolished when the mRNAs are freed from protein by phenol extraction. mRNAs isolated from the prestained polysomes exhibit normal activity in translation and in reverse transcription. This suggests that the dye may act by stabilizing or activating ribosomal protein factors involved in translation.

2. MATERIALS AND METHODS

Adult (250 g), male, Sprague-Dawley rats were used for the preparation of brain and liver polysomes. Coomassie brilliant blue R-250 was from British Drug Houses, Montreal. L-[4,5-^{3}H]Leucine (180 Ci/mmol) and [α-^{32}P]dATP (2800 Ci/mmol) were from Amersham, Ontario.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

Rabbit reticulocyte lysate was from Bethesda Research Laboratories, Gaithersburg, MD. AMV reverse transcriptase (16 U/μl) was purchased from Life Sciences, FL.

2.1. *Preparation of polysomes*

The tissues were homogenized in 10 vols of 0.25 M buffered sucrose (buffer: 50 mM Hepes-K^+, 100 mM K-acetate, 5 mM Mg-acetate, 5 mM β-mercaptoethanol, and 500 μg/ml of heparin, pH 7.4). The homogenate was centrifuged in a Sorvall SS-34 rotor at 5000 rpm for 10 min. Sodium deoxycholate and Nonidet P-40 were added to the supernatant to a final concentration of 1% each and the mixture was stirred for 10 min in an ice bath. Insoluble material was removed by centrifugation in the SS-34 rotor at 15000 rpm for 15 min. Aliquots of this supernatant were layered over equal volumes of 2 M buffered sucrose (buffer as in homogenization medium except that heparin was omitted), and the tubes were centrifuged in a Beckman SW 40 rotor at 40000 rpm for 4 h.

2.2. *Staining of polysomes with Coomassie blue*

Polysomes were suspended (50 A_{260} units/ml) in 0.25 M sucrose in gradient buffer (25 mM Hepes-K^+, pH 7.2, 4 mM Mg-acetate). A 0.4% solution of Coomassie blue was prepared by dissolving the dye in the same buffered sucrose used for the polysomes and removing any undissolved particles by centrifugation. Equal volumes of Coomassie blue and polysome suspensions were mixed and kept at 4°C for 1 h.

2.3. *Sucrose density gradient centrifugation*

Polysome suspensions (0.5–1.0 ml) were layered on 20–45% linear sucrose density gradients prepared in gradient buffer. The gradients were centrifuged at 27000 rpm for 4:5 h in a Beckman SW 27 rotor at 4°C. The distribution of polysomes on the gradients was recorded by using an ISCO recording spectrophotometer equipped with a flow cell and set at 254 nm.

2.4. *Preparation of mRNA*

mRNA was prepared as in [8] by affinity chromatography of total polysomal RNA on oligo(dT)-cellulose.

2.5. *Protein synthesis in vitro*

Protein synthesis was measured using the rabbit reticulocyte lysate in vitro translation system in the presence of either polysomes (3 mg/ml) or polysomal mRNA (40 μg/ml). The reaction was carried out according to the instructions provided by the manufacturer (BRL), using L-[^{3}H]leucine as tracer. The reaction mixtures were incubated at 30°C for 30 min.

2.6. *Synthesis of neuron-specific enolase (NSE) in vitro*

The reaction conditions were the same as for the determination of total protein synthesis except that, at the end of incubation, the newly synthesized NSE protein was recovered by immunoprecipitation and analyzed as described for S-100 protein [9]. NSE was isolated from brain according to Suzuki et al. [10]. Antibodies against the protein were prepared and purified as described [11].

2.7. *Reverse transcription of mRNA*

mRNA (1 μg) was incubated at 62°C for 2 min with 5 μl Tris-KCl (0.5 M Tris, 0.7 M KCl, pH 8.3). The mixture was cooled in ice water, and after addition of 2 μl oligo(dT) (1.25 mg/ml), reincubated at 42°C for 10 min. The rest of the analytical protocol was as described by Maniatis et al. [12]. [α-^{32}P]dATP was used as the tracer.

3. RESULTS

Fig.1 shows the blue bands obtained by sucrose density gradient centrifugation of brain polysomes previously stained with Coomassie blue. Although the bands were clearly visible against a faint background of blue color, it was difficult to photograph them in black and white because of lack of photographic contrast. The bands of polysomes could easily be recovered by puncturing the bottom of the tube with a hypodermic needle and collecting the drops corresponding to each band separately. Alternatively, the gradient could be monitored, as for unstained polysomes, by passage through the flow cell of a recording spectrophotometer set at 254 nm (fig.2).

The ultraviolet absorbance profile showed a good resolution of brain polysomes with narrow peaks and separation of aggregates of up to 14

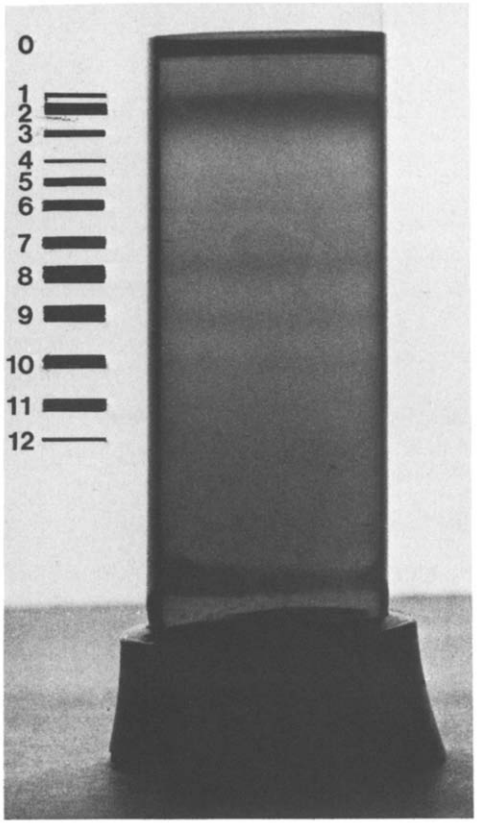

Fig.1. Banding of Coomassie blue-stained brain polysomes in sucrose density gradient. Positions of visible bands are indicated by dark lines to the left. The zero corresponds to the top of the gradient where the sample was applied.

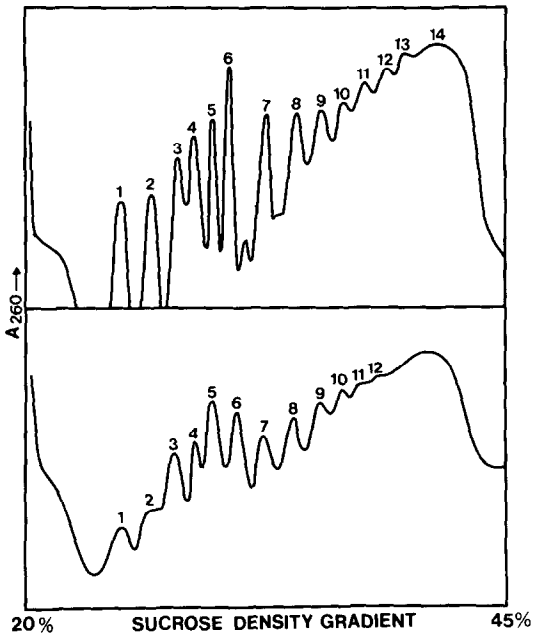

Fig.2. Ultraviolet absorption profiles of untreated (top) and Coomassie blue-stained (bottom) brain polysomes following sucrose density gradient centrifugation.

ribosomes (fig.2). Staining of these polysomes with Coomassie blue resulted in less compact peaks, although polysomal aggregates containing up to 12 ribosomes were distinguishable. A small fraction of polysomes formed an insoluble precipitate with Coomassie blue and sedimented to the bottom of the tube, thus causing a partial loss of polysomes from the gradient. Rat liver polysomes also behaved similarly on treatment with the dye.

Brain polysomes were more active in protein synthesis than liver polysomes (table 1) and this higher activity was exhibited by polysomal aggregates of all sizes ranging from trimers up to decamers and higher. Staining of polysomes with Coomassie blue enhanced their capacity for protein synthesis. This was true for both brain and liver polysomes and for each of the different size classes of polysomes.

We reported in [11] that the mRNA coding for NSE was present only in brain polysomes and in aggregates larger than pentamers. The results presented in table 2 confirmed these findings and further showed that brain polysomes stained with Coomassie blue were more active than untreated polysomes in the synthesis of NSE. Liver polysomes, as expected, did not synthesize NSE either with or without pretreatment with Coomassie blue.

mRNAs were isolated from untreated and Coomassie blue-stained brain polysomes. The 2 mRNAs were very similar in their ultraviolet spectra (not shown) and no residual Coomassie blue was detected in mRNA from stained polysomes. When the mRNAs were tested in vitro, they were all found to be active in the synthesis of total protein or of NSE and also of cDNA in the presence of AMV reverse transcriptase (table 3). However, no significant quantitative differences were observed between the 2 mRNAs with regard to any of these activities.

Table 1

Protein synthesis in vitro by polysome aggregates of different sizes separated by sucrose density gradient (SDG) centrifugation

Polysome peaks pooled from SDG (fig.2)	Brain polysomes		Liver polysomes	
	Untreated (control)	Prestained with Coomassie blue	Untreated (control)	Prestained with Coomassie blue
Peaks 1–3	26.2	185.2	35.6	52.5
Peaks 4 + 5	199.0	262.5	106.3	130.7
Peaks 6 + 7	271.5	386.3	188.7	238.3
Peaks 8 + 9	338.0	438.4	227.5	310.0
Peaks 10 and higher	309.0	460.2	214.0	263.8

Results are expressed as [^{3}H]leucine incorporated into protein (cpm $\times 10^{-3}$) in 25 μl reaction

Table 2

Synthesis of NSE by polysomes prestained with Coomassie blue

Polysome peaks pooled from SDG (fig.2)	Brain polysomes		Liver polysomes	
	Untreated (control)	Prestained with Coomassie blue	Untreated (control)	Prestained with Coomassie blue
Peaks 1–5	709	882	44	82
Peaks 5 and higher	2568	3250	102	145

Results are expressed as [^{3}H]leucine incorporated into NSE (cpm) in 50 μl reaction

Table 3

Translation and reverse transcription of brain polysomal mRNAs

	mRNAs isolated from	
	Untreated polysomes	Coomassie blue-stained polysomes
Total protein (25 μl reaction)	214562	198514
NSE (50 μl reaction)	2462	2618
cDNA (25 μl reaction)	235755	241687

Results are expressed as cpm incorporated

4. DISCUSSION

Our results reveal that: (i) polysomes stained with Coomassie blue retained their blue coloration during sucrose density gradient centrifugation and formed well-separated blue bands. (ii) The stained polysomes were not only active in protein synthesis, including synthesis of a tissue specific protein (NSE), but this activity was enhanced by staining. (iii) mRNAs isolated from these polysomes were fully active in translation and reverse transcription, but there were no differences between mRNAs of stained and control polysomes.

A number of dyes have been used for prestaining of proteins in attempts to visualize the migration

of protein molecules during electrophoresis and to recover the separated bands. The nature of the protein-dye interaction may influence the migration of proteins in an electrical or gravitational field. For example, Remazol, an amphoteric dye, does not cause changes in the net charge of proteins and consequently produces little change in electrophoretic migration [13,14]. On the other hand, prestaining with dyes such as bromphenol blue, bromphenol red or bromcresol green leads to a spreading of protein bands in polyacrylamide gels [15]. Charge effects may be expected to be less significant in centrifugal separations, unless they also bring about simultaneous modifications in protein conformation and changes in volume due to altered hydration of the sedimenting particles. Coomassie blue is a triphenylmethane dye, belonging to the magenta family [16]; it binds to proteins by reversible electrostatic interactions, the complex being further stabilized by Van der Waals forces [2]. The similarity of the ultraviolet absorbance profiles of stained and control polysomes (fig.1) would indicate that Coomassie blue staining did not produce conformational or other modifications large enough to affect significantly the sedimentation behavior of polysomes.

In view of the fact that Coomassie blue specifically interacts with proteins, the enhanced protein synthetic activity by stained polysomes may be attributed to the possible action of this dye: (i) in protecting the polysomes against degradation by endogenous or exogenous nucleases during their preparation and testing; or (ii) in protecting or activating the protein factors present in polysomes and required for initiation or elongation of polypeptide chains. The observation that the positive effects of Coomassie blue were confined to polysomes and disappeared once the mRNAs were freed from proteins would appear to support the second hypothesis. We have shown previously that brain mRNA required the presence of tissue-specific initiation factors associated with homologous polysomes for optimum activity in vitro [17]. The favorable action of Coomassie blue when brain polysomes, and not brain mRNA, were translated in the heterologous reticulocyte lysate system would indicate that this dye may interact with the initiation protein factors in polysomes and stabilize them against inactivation.

ACKNOWLEDGEMENTS

This research was supported by grants from the Medical Research Council of Canada and the Institut National de la Santé et de la Recherche Médicale of France. We are grateful to the governments of Québec and France for help in developing an exchange program between our laboratories in Québec and Clermont-Ferrand.

REFERENCES

[1] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[2] Fazekas de St. Groth, S., Webster, R.G. and Datyner, A. (1963) Biochim. Biophys. Acta 71, 377–391.
[3] Kahn, R. and Rubin, R.W. (1975) Anal. Biochem. 67, 347–350.
[4] Diezel, W., Kopperschlager, G. and Hofman, E. (1972) Anal. Biochem. 48, 621–623.
[5] Reisner, A.H., Nemes, P. and Bucholtz, C. (1975) Anal. Biochem. 64, 509–516.
[6] Bertolini, M.J., Tankersley, D.L. and Schroeder, D.D. (1976) Anal. Biochem. 71, 6–13.
[7] Malhotra, L.C., Murthy, M.R.V. and Chaudhary, K.D. (1978) Anal. Biochem. 86, 363–370.
[8] Murthy, M.R.V. (1983) Neurochem. Int. 5, 395–403.
[9] Murthy, M.R.V. (1982) J. Neurochem. 38, 41–51.
[10] Suzuki, F., Umeda, Y. and Kato, K. (1980) J. Biochem. 87, 1587–1594.
[11] Murthy, M.R.V., Bharucha, A.D., Charbonneau, R. and Chaudhary, K.D. (1977) in: Mechanisms, Regulation and Special Functions of Protein Synthesis in Brain (Roberts, S. et al. eds) pp.21–28, Elsevier, Amsterdam, New York.
[12] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) in: Molecular Cloning: a Laboratory Manual, pp.229–234, Cold Spring Harbor Laboratory, NY.
[13] Griffith, I.P. (1972) Anal. Biochem. 46, 402–412.
[14] Datyner, A. and Finnimore, E.D. (1973) Anal. Biochem. 55, 479–491.
[15] Hattingh, J., Coetzec, N. and Ross, F.P. (1977) Anal. Biochem. 80, 635–638.
[16] Color Index, 2nd Ed. (1956) Am. Assoc. Textile Chemists Colorists, Lowell, MA.
[17] Murthy, M.R.V. (1983) Neurochem. Int. 5, 385–394.