Simultaneous quantification of several mRNA species by solution hybridisation with oligonucleotides

Michael C.O'Donovan, Paul R.Buckland and Peter McGuffin
Department of Psychological Medicine, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, UK

Submitted January 29, 1991

We have developed a solution hybridisation procedure for the assay of mRNA using end labelled oligonucleotide probes based on the method of S1 nuclease protection (1). This has a number of advantages over Northern blotting and other solution hybridisation procedures which use cDNA or RNA probes, including the facility to use several different probes simultaneously.

Measurement of mRNA levels has in the past principally been carried out using the technique of Northern blotting. However, recent advances in molecular biology have considerably increased the utility of the less commonly used technique of excess probe solution hybridisation, and protocols have been reported which use either single stranded cDNA probes made from M13 clones (2), or cRNA probes (3) of a single specific length. In this method radiolabelled nucleotide probe is hybridised directly to RNA in solution, increasing the sensitivity of the assay and removing the need for blotting. Following removal of unbound single stranded probe by specific enzyme digestion the remaining double stranded probe is assayed by polyacrylamide gel electrophoresis (PAGE) and densitometry. However, the use of oligonucleotide probes has several advantages over the alternatives. They are easily made and the possession of the corresponding cDNA is not required. They are simply labelled and purified, are highly specific for the target mRNA and sequences complementary to different regions of the target mRNA can be used. Also a large excess of probe can be used, due to the relative ease of removal, lowering the hybridisation time. A further major advantage is the ease with which a number of different length probes can be labelled and simultaneously hybridised with an RNA sample. Being of different lengths the protected fraction of each of the probes can be quantified after PAGE allowing multiple species of mRNA to be measured, including a reference probe such as beta-actin, in a single procedure.

Solution hybridisation is considered to be 10–20 times more sensitive than Northern blotting, but 5′ end labelled probes have a much lower specific activity than random primer labelled cDNA probes. Overall, therefore, the two procedures may be expected to have similar sensitivity, and this has been confirmed by direct comparison. Alternative methods have been described of labelling oligonucleotides to a greater specific activity if required (1, 4).

Oligonucleotide probes prepared 'in house' are end labelled with polynucleotide kinase using gamma ^{32}P-ATP using standard procedures. Probes and RNA are suspended in 30 μl of hybridization buffer (probe concentration 1 pmole/ml, 0.4 M NaCl, 40 mM pipes pH 6.4, 1 mM EDTA) heated at 90°C for 3 min and incubated at 70°C for 2 h. Excess probe is then removed by the addition of 300 μl of S1 nuclease buffer (S1 nuclease 120 U/ml, zinc sulphate 4.5 mM, sodium acetate 50 mM, pH 4.2, sodium chloride 0.3 M, 10 μg/ml single stranded DNA) and digestion occurs for 15 min at 37°C. 300 μl of the reaction mixture is transferred to a fresh tube and the reaction is terminated by the addition of 48 μl 4 M ammonium acetate, 0.1 M EDTA. Double stranded hybrids are precipitated with ethanol, resuspended in 20 μl formamide running buffer, denatured at 90°C for 2 min, subjected to acrylamide gel (10%) electrophoresis and analysed by autoradiography followed by scanning densitometry. Where more than one probe is used the specific activity of each probe may be adjusted empirically to give similar band intensities.

Using the above procedure, probes can be labelled, hybridised with RNA, excess probe removed and electrophoresis completed within eight hours. From the resulting autoradiograph, a number of different probes can be quantified relative to a reference probe in each of the lanes.

ACKNOWLEDGEMENTS

Supported by grants from The Medical Research Council, The Mental Health Foundation and The Schizophrenia Research Fund.

REFERENCES

1. Reyes,A.A. and Wallace,R.B. (1987) *Methods Enzymol.* **154**, 87–94.
2. Hadcock,J.J. and Malbon,C.C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5021–5025.
3. Chao,H.M., Choo,P.H. and McEwen,B. (1989) *Neuroendocrinol.* **50**, 365–371.
4. Gauthier,E., Chapdelaine,P., Tremblay,R.R. and Dube,J.Y. (1990) *Nucl. Acids Res.* **18**, 7450.

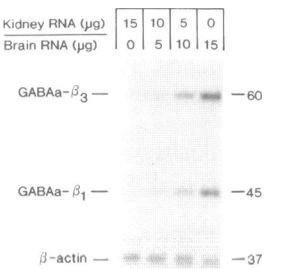

Figure 1. Autoradiograph of rat brain and kidney total RNA probed with end labelled oligonucleotides of indicated length complementary to the GABA-A beta 3 and beta 1 receptor subunits and beta-actin. Relative band intensities for G-ABAA-beta1 are 0, 10, 17 and 24.5 and for GABAA-beta3 are 0, 9, 16 and 23.5 for samples containing 0, 5, 10 and 15 μg of brain RNA respectively.