A SIMPLE METHOD FOR THE QUANTITATIVE ISOLATION OF UNDEGRADED HIGH MOLECULAR WEIGHT RIBONUCLEIC ACID.

J.J. Barlow, A.P. Mathias and R. Williamson
Department of Biochemistry, University College, London, W.C.1.

and D.B. Gammack, Institute of Psychiatry, Maudsley Hospital, Denmark Hill, London, S.E.5.

Received August 7, 1963

Curry and Hersh (1962) have reported that ribosomal protein may be isolated by treatment of a ribosome suspension with 2M-LiCl. We have applied this technique to rabbit reticulocyte ribosomes and found that it affords a method for the quantitative recovery of undegraded ribosomal RNA as well as the protein. The method can be extended in several ways and also permits a simplification of the standard method of isolation of s-RNA.

Experimental: Reticulocyte ribosomes were suspended in .05M-KCl, .001M-tris, pH 7.5, .0015M-$MgCl_2$ ("TKM buffer") at a concentration of from 2-20 mg./ml. and an equal volume of 4M-LiCl added. The solution was kept at 4°C for 16 hours. It was found in agreement with Curry and Hersh (1962) that ribosomal protein was quantitatively solubilized (the contamination with RNA, estimated by the orcinol method, was less than 1.5%, the limit of the sensitivity of the assay). An investigation of reticulocyte ribosomal protein prepared in this manner will be reported (Mathias and Williamson, in preparation).

The precipitate was isolated by centrifugation at 3,000 rpm for two minutes, and was washed twice by resuspension in 2M-LiCl followed by recentrifugation. The recovery of RNA was quantitative (contamination with protein, estimated by the Lowry method, after washing and dissolving was less than 1%).

The RNA was dissolved with the aid of one or two thrusts by hand of a wide clearance (.012") Potter homogenizer in .02M-sodium phosphate buffer, pH 6.8, with or without .001M-EDTA. The concentration of RNA was from 0.5-5.0 mg./ml. The ultraviolet absorbtion spectrum of the RNA is shown in Fig. 1. There is a maximum at 257 mμ; a minimum at 231 mμ; the ratio of the optical densities at 280 mμ/260 mμ is 0.49. The optical density at 260 mμ of a solution containing 1 mg./ml. RNA is 23.8.

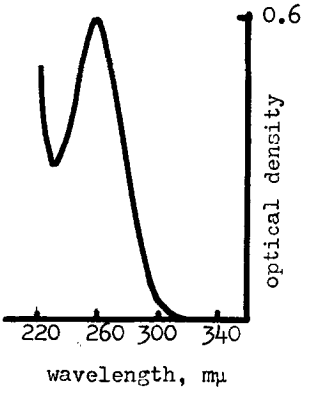

Fig. 1. Absorbtion Spectrum of LiCl-isolated RNA

The clean separation of RNA from protein using ribosomes suggested that this method might be applicable to cruder preparations. An equal volume of 4M-LiCl was added to the supernatant from a suspension of rabbit reticulocytes which had been lysed with 3 volumes of .001M-$MgCl_2$ and then centrifuged at 20,000g for 5 minutes. During storage at 4°C for 16 hours a white gelatinous precipitate formed which can be isolated, washed and redissolved as above. Analysis of the redissolved precipitate showed that it contained only RNA. The spectrum is identical to that of the RNA isolated from ribosomes. The recovery is quantitative for ribosomal RNA, representing some 80-85% of the total RNA of the lysate.

RNA isolated by this procedure from ribosomes (RNA "Rb") or from lysate (RNA "L") was dissolved in 0.02M-phosphate buffer, pH 6.8, and the solutions were investigated using the Spinco model E analytical ultracentrifuge.

Both preparations gave identical sedimentation patterns of three components. Sedimentation coefficients were measured for the three components and the values obtained after extrapolation to infinite dilution ($S^{o}_{20,w}$) were 17s, 28s and 41s. The sedimentation pattern is shown in Fig.2, taken 18 minutes after reaching speed, 42,040 rpm.

Fig. 2. Analytical Ultracentrifuge Trace of LiCl-isolated RNA

Sedimentation analysis by density gradient centrifugation, using the technique of Edwards and Mathias (1963), which enables the position of the peaks on the gradient to be visualized directly using a recording spectrophotometer, gives a similar picture. 0.1 mls of an RNA solution (containing approximately 1 mg./ml. RNA) was layered on a 5-20% sucrose gradient containing .02M-phosphate buffer, pH 6.8. The sucrose had been previously treated with small amounts of bentonite to inactivate ribonuclease (RNase) which contaminates even Analar grade sucrose. The gradient tubes were centrifuged at 37,000 rpm for from 2-4 hours in the SW 39 swing out rotor of the Spinco model L ultracentrifuge. The patterns obtained for RNA "Rb" and RNA "L" are shown in Figures 3 and 4. In a similar experiment the RNA was dissolved in buffer containing .001M-EDTA, and centrifuged through a gradient also containing EDTA. The pattern remained identical, showing that the presence of the 41s peak is independent of the presence of chelating agents.

The relative proportions of the three peaks are approximately as follows:

41s, 2% 28s, 67% 17s, 31%

s-RNA is not precipitated by lithium chloride under the conditions of the extraction, but can be extracted by the phenol method of Kirby (1956) from the LiCl-soluble fraction after precipitation of the ribosomal RNA. The sedimentation pattern of such a phenol preparation, under similar conditions of density gradient centrifugation to those outlined above, is shown in Fig.5. Only one RNA component is found, sedimenting at approximately 4s. Phenol extraction of the reticulocyte post-mitochondrial supernatant gives three peaks of approximate sedimentation coefficients 4s, 16s and 28s.

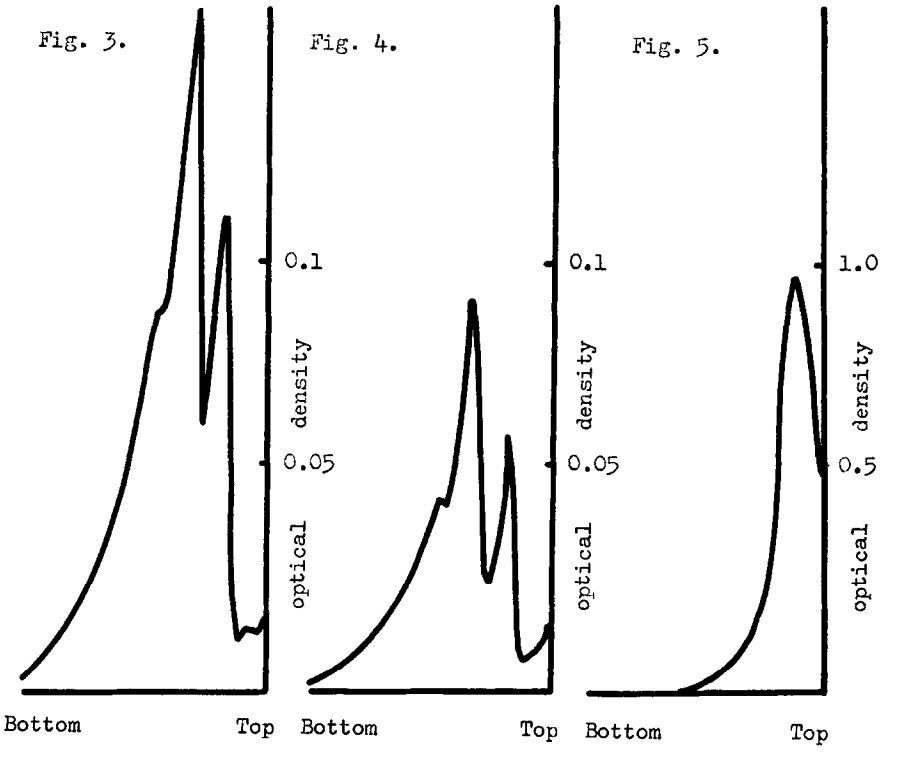

Fig. 3. Sucrose gradient trace RNA "Rb"
Fig. 4. Sucrose gradient trace RNA "L"
Fig. 5. Sucrose gradient trace: phenol-extracted RNA from LiCl supernatant

This method may prove useful in isolating s-RNA. The usual methods for preparing s-RNA involve a preliminary centrifugation for several hours; this can be eliminated by carrying out phenol extraction directly on the supernatant of a LiCl-treated post-mitochondrial supernatant.

Discussion: It has been reported (Tashiro et.al., 1960) that high molecular weight RNA precipitated using concentrated salt solutions suffers considerable degradation. It is apparent from the above results that in the case of reticulocyte RNA precipitated by the LiCl technique, no such degradation occurs. This may be related to the fact that reticulocyte ribosomes contain only minute traces of latent RNase (Williamson and Mathias, 1963); however, RNase is found in the post-mitochondrial supernatant.

The two major peaks isolated by precipitation with LiCl (17s and 28s) correspond to the peaks usually isolated from ribosome preparations (Spirin, 1963). The minor component sedimenting at 4ls may be identical with the rapidly labelled RNA component of similar sedimentation coefficient isolated from nucleated animal cells, thought to be messenger RNA or a precursor of ribosomal RNA (Scherrer et.al., 1963). The fact that such a component is not found in the RNA isolated by the phenol technique in our system may be due to its concentration at the interphase, as reported by Sibitani et al (1962) for this isolation procedure. If undegraded messenger RNA can be isolated in good yield by the LiCl procedure outlined above, this should stimulate investigations into its properties. Preliminary results indicate that the technique is equally successful with nucleated reticulocytes as with enucleate rabbit reticulocytes. We are investigating the effect of adding the various fractions of RNA isolated by the above technique to cell-free protein synthesis systems with varying polysome content (Mathias and Williamson, in preparation).

We wish to thank the British Empire Cancer Campaign for a personal and material grant to one of us (R.W.) and the Department of Scientific and

Industrial Research (G.B.) for a personal grant to one of us (J.J.B.).

We are grateful to the Medical Research Council for providing the

Spinco analytical ultracentrifuge.

References.

Curry and Hersh, Biochem.Biophys.Res.Comm., 6, 415 (1962).
Edwards, J.G. and Mathias, A.P., Nature, in press.
Kirby, K.S., Biochem.J., 64, 405 (1956).
Scherrer, K., Latham, H., Darnell, J.E., Proc.Nat.Acad.Sci. U.S., 49, 240 (1963
Sibatani, A., De Kloet, S.R., Allfrey, V.G. and Mirsky, A.E., Proc.Nat.Acad.Sci U.S., 48, 471 (1962).
Spirin, in "Progress in Nucleic Acid Research" ed. Davidson and Cohn, Vol.1, p.301 (1963).
Tashiro, Y., Shimidzu, H., Inouye, A., and Kakiuchi, K., Biochim.Biophys.Acta, 43, 544 (1960).
Williamson, R. and Mathias, A.P., Biochem.J. (Proceedings), in press.