Electrophoresis in Polyacrylamide Gels of Different 'Soluble' Ribonucleic Acid Preparations from *Escherichia coli*

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Several methods have been used to prepare s-RNA* from micro-organisms, usually involving either direct phenol extraction of whole cells (Holley, 1963) or phenol extraction of a ribosome-free supernatant fraction (Tissières, 1959). Some techniques have involved only protein removal, and these preparations are usually heterogeneous and may contain several minor RNA components having no transfer function (Brown, 1963; Richards & Gratzer, 1964). Only further purification by methods such as the use of Sephadex G-100 (Schleich & Goldstein, 1966a) will result in the isolation of uncontaminated t-RNA.

We have examined by electrophoresis in polyacrylamide gels s-RNA preparations from *Escherichia coli* obtained both from whole cells and supernatant fractions by using modifications of the methods of Holley (1963) and Tissières (1959). Such s-RNA preparations, when analysed by sucrose-density-gradient centrifugation as described by Cannon, Krug & Gilbert (1963), sediment as single peaks with no apparent contamination from other RNA. We now show, however, that, though supernatant preparations show exclusively one component on gel electrophoresis, whole-cell preparations yield mainly two components, the second of which derives entirely from the ribosomes.

E. coli (strain B163) was grown, harvested and washed as described by Cannon (1966). s-RNA was isolated by two different methods. In the whole-cell method, cells were harvested and washed and then suspended in water (2g. wet wt. in 15ml.). The suspension was then mixed with an equal volume of phenol (90%, w/v) (Kirby, 1956) and shaken for 60min. at room temperature. The mixture was centrifuged to separate the two phases and the top aqueous layer was collected. The phenol layer was extracted for a further 15min. with an equal volume of water and centrifuged. The combined aqueous layers from the two extractions were centrifuged (700g) to remove traces of phenol, 0.2vol. of 5M-NaCl was added and the RNA was precipitated with 2vol. of 95% (v/v) ethanol. The RNA was collected

by centrifugation and extracted with 5ml. of ice-cold M-NaCl. The extract was centrifuged, the supernatant liquid collected and the pellet extracted twice more with 5ml. portions of ice-cold M-NaCl. RNA was precipitated from the combined supernatant fractions with 2vol. of 95% ethanol and dissolved in water, any insoluble material being removed by centrifugation. The RNA was then applied to a DEAE-cellulose column and the s-RNA eluted as described by Cannon (1966).

In the supernatant method s-RNA was prepared from frozen *E. coli* cells as described by Cannon *et al.* (1963). The RNA obtained by phenol extraction of the supernatant fraction was purified further on DEAE-cellulose as described above.

Ribosomes in suspension in 5mM-tris-HCl buffer, pH 7.2, containing magnesium acetate (10mM), and previously washed four times in this buffer, were prepared as described by Cannon *et al.* (1963). RNA from these preparations was obtained by treating the ribosomes with 0.5% sodium dodecyl sulphate for 5min. at room temperature followed by phenol extraction and DEAE-cellulose purification as described above.

The various RNA samples were analysed by electrophoresis on polyacrylamide gels with the cacodylate-aminazole buffer system at pH 7.2 described by Richards, Coll & Gratzer (1965). The results are given in Fig. 1, which shows densitometer tracings of the gels. Fig. 1(a) illustrates the behaviour of the whole-cell preparation. From the area under the curves on the densitometer tracings we calculate that about 96% of the material is found in two main bands *A* and *B*, comprising respectively 75% and 21% of the total. From its R_F value, component *A* corresponds to mixed t-RNA and all the amino acid-acceptor activity is concentrated in this band (Richards *et al.* 1965). Component *B*, which possesses no acceptor activity, was identified as 5s RNA from the ratio of its mobility to that of t-RNA (J. Hindley, personal communication). A minor band is also present with a mobility lower than that of 5s RNA and this may correspond to a similar component obtained by J. Hindley (personal communication). The relative amounts of t-RNA (75%) and 5s RNA (21%) do not necessarily

* Abbreviations: s-RNA, 'soluble' RNA; t-RNA, transfer RNA.

Fig. 1. Densitometer tracings of polyacrylamide gels. Samples of s-RNA were prepared and analysed by electrophoresis on polyacrylamide gels as described in the text: R_s , ratio of distance migrated relative to the anion front. (a) s-RNA prepared by the whole-cell method; (b) s-RNA prepared by the supernatant method; (c) s-RNA prepared from ribosomes.

represent their ratio within the cell, as whole-cell extraction is not designed as a quantitative technique and may leach out RNA components preferentially. To support this, phenol extraction of cells previously frozen yields more ribosomal RNA than extraction from fresh unfrozen cells without significant change in amounts of total extractable s-RNA.

Fig. 1(b) shows the behaviour of the supernatant preparation. Essentially all the material travels with the mobility of the A band, the gel being uncontaminated with the B component. This preparation therefore seems to contain only mixed t-RNA.

As a control we examined by gel electrophoresis the low-molecular-weight RNA from ribosomes. The pattern obtained is illustrated in Fig. 1(c). As in Fig. 1(a) there are two major components A and B, and we may thus deduce that component B as present in whole-cell preparations of s-RNA

derives entirely from the ribosomes. This result is similar to the one obtained by J. Hindley (personal communication), although our ribosome preparations contain considerably more t-RNA than he found. This difference is almost certainly due to the different washing procedures used in the preparations.

In our ribosomal preparations components A and B are present in about equimolar proportion. Rosset, Monier & Julien (1964) have shown that 5s RNA is attached to the 50s sub-unit of *E. coli* ribosomes, and Zehavi-Willner & Comb (1966) have calculated that each 50s particle contains two molecules of 5s RNA. A similar value has been reported by Schleich & Goldstein (1966b). Hence, if we assume that our ribosome preparations also have this 5s content, there are in addition approximately two molecules of t-RNA (band A) for each molecule of 50s ribosome, a value that agrees with that reported by Cannon *et al.* (1963) for ribosomes prepared in this way.

We also studied the electrophoretic behaviour of whole-cell and supernatant preparations before the DEAE-cellulose purification stage, a procedure that is included to remove ribosomal RNA and small-molecular-weight impurities. In both cases the gel patterns were similar to those illustrated in Fig. 1(a) for the DEAE-cellulose-purified samples, except that with the whole-cell preparations considerable amounts of ribosomal RNA were present which remained at the top of the gel. As expected, there was no ribosomal RNA in the supernatant preparations.

While this manuscript was in preparation a paper appeared by King (1967), who studied the heterogeneity of rat-liver s-RNA preparations, and some of our results are similar to his. However, it is apparent that the s-RNA preparations we have obtained from *E. coli* contain far fewer components. Further, the purest s-RNA preparation he obtained contained only 78% of t-RNA.

In conclusion, the main difference between the two methods of s-RNA isolation discussed here resides in the presence of the 5s component in whole-cell preparations. This derives entirely from the ribosomes, but is not retained by DEAE-cellulose as is normal ribosomal RNA. Isolation of s-RNA from supernatant preparations yields a homogeneous product of mixed t-RNA with contaminants suppressed to an inconspicuous level.

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Loss of Coding Properties of the Oligonucleotide Adenylyluridylylguanosine after Photoreduction or Hydration

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Madison & Holley (1965) first established the existence of 5,6-dihydrouridylic acid in s-RNA* and evidence obtained by Huang & Bonner (1965) and by Carr & Grisolia (1964) suggests that it may be a component of other types of RNA. Mokrasch & Grisolia (1959) discovered dihydropyrimidine dehydrogenases that reversibly interconvert uridylic acid and dihydrouridylic acid. These observations indicate that dihydrouridylic acid may have a special role in protein synthesis.

Brown, Lee & Metz (1966) have suggested that the presence of one or more dihydrouridylic acid residues in a viral messenger RNA together with the existence in the virus-infected cell of a nucleotide-sequence-specific dihydrouridine dehydrogenase might constitute a translational control mechanism for viral protein synthesis. This postulated inter-conversion of uridylic acid and dihydrouridylic acid in messenger RNA would be expected to have a functional effect because the properties of uridylic acid are altered considerably when the 5,6-double bond is saturated to give either dihydrouridylic acid

or the closely similar 5-hydro-6-hydroxyuridylic acid. It has been shown that dihydrouridylic acid residues in poly(U,H₂U) copolymers are unable to form a helical structure with polyA (Cerutti, Miles & Frazier, 1966), and Rottman & Cerutti (1966) and Grossman (1963) have demonstrated that the partial conversion of uridine into dihydrouridine or into 5-hydro-6-hydroxyuridine in polyU decreased the incorporation of phenylalanine. However, though Smrt, Škoda, Lisý & Šorm (1966) found that the trinucleoside diphosphate GpUpU was unable to bind valyl-s-RNA if any or both of the uridine residues were replaced by dihydrouridine, the results of Rottman & Cerutti (1966) suggested that the binding of phenylalanyl-s-RNA was not sensitive to the amount of dihydrouridylic acid in copolymers of uridylic acid and dihydrouridylic acid.

It has recently been established that *N*-formyl-methionine acts as a chain initiator for protein synthesis in *Escherichia coli* cells (Clark & Marcker, 1966; Adams & Capecechi, 1966; Webster, Engelhardt & Zinder, 1966) and it is therefore of particular interest, in relation to possible control mechanisms, to investigate the effect of altering the codon for *N*-formylmethionine. The effect of u.v. irradiation on the codon is also of interest in the context of radiation damage. In this paper we show that when the uridylic acid in ApUpG, which is a coding triplet for *N*-formylmethionine (Clark & Marcker, 1966; Kellogg, Doctor, Loebel & Nirenberg, 1966), is replaced by dihydrouridylic acid, or the hydrate, it is no longer capable of specifically binding *E. coli*

* Abbreviations: s-RNA, 'soluble' or transfer RNA; A, adenosine; U, uridine; G, guanosine; H₂U, 5,6-dihydrouridine; (H₂O)U, 5-hydro-6-hydroxyuridine. Oligonucleotides have been written with a p to the right to indicate a 3'-phosphate, e.g. ApH₂UpG for adenylyl-(3'-5')-dihydrouridylyl-(3'-5')-guanosine, and codons have been abbreviated to the nucleosides in sequence. The constituent nucleosides have been used to designate polyribonucleotides, e.g. polyU for polyuridylic acid, poly(U,H₂U) for a copolymer of uridylic acid and dihydrouridylic acid of random sequence.