THE PREPARATION OF RIBOSOMAL PROTEIN FROM ESCHERICHIA COLI WITH LITHIUM CHLORIDE AND UREA

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The three methods most widely used for the preparation of ribosomal proteins from Escherichia coli are the following: extraction with 66% acetic acid, (Waller and Harris, 1961) dialysis against 6M urea, (Spahr, 1962) and dialysis against 1M salt (Spitnik-Elson, 1962). The first method directly separates protein from RNA. In the latter two techniques the RNA is degraded by the ribosomal ribonuclease and removed by dialysis, leaving the protein free in solution. The starch gel electrophoresis patterns of proteins prepared by these methods are complex. Furthermore, different isolation procedures have not always given proteins with the same electrophoretic patterns (Spahr, 1962). Since this suggests that the isolation procedure itself may be a factor in the observed heterogeneity of the proteins, we have tried to apply a fourth method of preparation in order to investigate this point.

The proteins of yeast (Chao, 1961) and reticulocyte (Mathias and Williamson, 1964) ribosomes have been prepared by exposing the ribosomes to 2M LiCl. This reagent precipitates the RNA, while the ribosomal proteins remain in solution. The ribosomes of E. coli do not behave in the same way. The RNA of these ribosomes precipitates neither in 2M LiCl nor in higher

concentrations, up to 5M. We have, however, succeeded in precipitating the ribosomal RNA by adding urea to the LiCl solution. A series of experiments has shown that there is virtually complete precipitation of the ribosomal RNA and complete solubilization of the protein in a solution containing 3M LiCl and 4M urea. The procedure is described in this communication.

Methods

Protein was determined with the Folin reagent, with crystalline bovine serum albumin (Armour) as standard.

Phosphorus was assayed according to King, (1932) but with the following modification, taken from Bartlett's (1959) procedure. After addition of the reagents, the samples were heated in boiling water for seven minutes. The intensity of the color produced was measured at 830 mμ. This modification increases the sensitivity several times.

Total RNA was determined in two ways: with the orcinol reaction against a standard of analyzed yeast RNA, and by the optical density of total alkaline hydrolysates. In the latter procedure a sample was digested with 0.4M NaOH at 37° for 18 hours and brought to pH 7 by the addition of over two volumes of 1M sodium phosphate buffer of pH 7.0. RNA concentration was computed from the difference in optical density at 260 mμ and 290 mμ with a factor calculated for *E. coli* ribosomal RNA.

Experimental

The ribosomal proteins are prepared in the following way. A solution of 6M LiCl and 8M urea is added to an equal volume of ribosomes prepared according to Tissières et al.

(1959) and dissolved in 0.01M magnesium acetate and 0.001M Tris-HCl buffer of pH 7.4. The solution is mixed well and left at 4° for 24 hours. The resulting precipitate is separated by centrifugation at 6000 RPM for 10 minutes at 4°. It is washed twice with 3M LiCl-4M urea and the washings are added to the original supernatant. To this solution, which contains all of the ribosomal protein, is added an equal volume of cold 20% trichloroacetic acid, and the mixture is left overnight at 4°. The precipitated

TABLE 1

THE ISOLATION OF RIBOSOMAL PROTEINS FROM E. COLI

	Ribosomes	RNA	Protein		
	Original solution	LiCl-urea precipitate	LiCl-urea supernatant precipitated with trichloroacetic acid and taken up in 0.01M HCl		
			Before centrifugation	After centrifugation	
				Supernat.	Pellet
Volume (ml)	4.0	25.0	25.0	25.0	1.5
Total quantity (mg)					
Phosphorus	4.8	4.3	0.255	0.08	0.12
RNA (orcinol)	62.8	59.0	2.25	0.375	1.00
RNA (U. V.)	58.0	51.0	-	-	-
Protein	49.0	0	50.0	46.25	2.7
Percent					
Phosphorus	100	89.6	5.3	1.7	2.5
RNA (orcinol)	100	93.9	3.6	0.6	1.6
RNA (U. V.)	100	87.9	-	-	-
Protein	100	0	102.0	94.4	5.5

proteins are centrifuged down, washed three times with ether, suspended in 0.01M HCl, and dialyzed against the same solvent for 24 hours at 4°.

The protein solution so obtained is slightly turbid. It contains all of the ribosomal protein and about 5 - 6% of the phosphorus of the ribosomes. Centrifugation at 10,000 RPM for 30 minutes at room temperature gives a clear solution which contains about 94% of the ribosomal protein and about 1 - 2% of the ribosomal phosphorus. The precipitate contains about 5 - 6% of the protein and about 3% of the phosphorus. The results of a typical preparation are summarized in Table 1.

Discussion

The method gives a good recovery of the ribosomal protein, without recourse to the ribonuclease digestion of the ribosomal RNA. Apparently, only the ribosomal RNA precipitates in the LiCl-urea solution. This precipitate contains only 90% of the total ribosomal phosphorus. Of the remaining 10%, about half is lost during the trichloroacetic acid precipitation of the proteins and their subsequent dialysis against 0.01M HCl. Even after prolonged dialysis at pH 2, however, the proteins still contain phosphorus. Furthermore, the solution is slightly turbid, although from their amino acid composition, the proteins should be soluble at this pH, all being positively charged. This suggests that a macromolecular RNA might be present, possibly transfer RNA, which is soluble in concentrated salt solutions but insoluble at pH 2. When this turbid solution is centrifuged, the clear

supernatant, with 94 - 95% of the protein, still contains about 1.5% of the ribosomal phosphorus.

It should be noted that the RNA content of this clarified protein solution, as determined with the orcinol reagent, is only 0.6% of the ribosomal RNA. E. coli ribosomal proteins prepared by acetic acid extraction or by RNase digestion in 6M urea and assayed in the same way are reported to have the same RNA content. Phosphorus was not determined in those preparations, but the phosphorus content of the clear protein solution described in this communication is too high to be entirely accounted for by the quantity of RNA as determined by the orcinol reaction. It is possible, that the differences in the electrophoretic patterns of ribosomal proteins which have been prepared in different ways might be due to phosphorus containing non-protein contaminants which are attached to or interact with the proteins. This might also conceivably be one factor which contributes to the observed electrophoretic heterogeneity of the proteins.

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