ASSOCIATION OF RIBOSOMAL RNA WITH RIBOSOMAL PROTEINS COVALENTLY BOUND TO SEPHAROSE

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1. Introduction

Solid-matrix bound proteins have won wide application in studying biological mechanisms and interactions (for ref., see [1]). Insoluble enzymes, antigens and other biologically active proteins preserve their activity for a long time, and can be used repeatedly. This may be especially valuable in the case of individual ribosomal proteins whose isolation in preparative amounts is still a difficult problem. This paper describes the preparation of Sepharose-bound ribosomal proteins and estimation of their ability to form specific complexes with ribosomal RNA.

2. Materials and methods

Crude 70 S ribosomes were prepared from *E. coli* MRE 600 by a modified method of Tissières and Watson [2]. Isolated 30 S and 50 S subunits were prepared by centrifugation in a BXXX-A zonal rotor [3]. Total 30 S ribosomal protein was extracted from 30 S particles with 4 M urea–2 M LiCl solution. Individual ribosomal proteins were prepared by column chromatography on phosphocellulose (Mann) as described by Hardy et al. [4]. Isolated proteins were identified by polyacrylamide gel electrophoresis. 23 S and 16 S RNA's from 30 S and 50 S ribosomal subunits were prepared by the standard procedure of phenol–sodium dodecylsulphate deproteinization. Cyanogen-bromide-activated Sepharose 2B (Pharmacia, Sweden) was used for fixation of ribosomal proteins. Activation of the polysaccharide was performed according to Axén et al. [5]. The coupling reaction of ribosomal proteins with activated Sepharose was allowed to proceed in 10^{-2} M potassium phosphate – 10^{-2} M magnesium acetate – 1.0 M KCl, pH 7.8 buffer, at 4° for 24–40 hr. The polymers were washed with the same buffer and stored at 4°. To check the specific RNA-binding activity polymer-bound proteins were mixed with 16 S or 23 S RNA under the conditions proposed by Traub and Nomura for reconstitution of 30 S subunits (10^{-2} M potassium phosphate – 10^{-2} M magnesium acetate – 0.3 M KCl standard buffer pH 7.8, 40°, 30 min) [6] and the Sepharose was vigorously washed to remove non-complexed RNA. To elute the RNA which became attached to protein, the concentration of KCl in the buffer was raised to 1.0 M and RNA concentration in the solution was measured.

3. Results and discussion

The results presented in table 1 show that cyanogen-bromide-activated Sepharose binds the ribosomal proteins in a good yield. The coupling reaction depends upon the kind of protein used. The highest coupling yield (80%) was obtained with low molecular weight, strongly basic protein S20 (here and below the nomencalture suggested by Wittmann et al. [7] is used). It is very important that under coupling reaction conditions no binding of ribosomal RNA to activated Sepharose occurs.

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Table 1
Chemical fixation of ribosomal proteins to cyanogen-bromide-activated Sepharose 2B.

Protein or RNA	Amount of protein in the reaction mixture added to 1 ml Sepharose (mg)	Amount of fixed proteins (mg/ml)	Coupling yield (%)
Total 30 S ribosomal protein	0.90	0.47	52
S7	1.50	0.88	59
S20	0.25	0.20	80
16 S	0.3	0.0	0.0
23 S	0.3	0.0	0.0

Table 2
Complexing of ribosomal RNA with Sepharose-bound proteins.

Protein	RNA	Amount of RNA in complexes (nmoles/mg of protein)	Specificity factor
30 S	23 S	0.15	2
	16 S	0.50	
S7	23 S	0.04	3
	16 S	0.13	
S20	23 S	0.22	10
	16 S	2.12	

It is seen in table 2 that 30 S proteins after being fixed on Sepharose preserve to some extent their ability to bind 16 S RNA. It should be noted, however, that insoluble 30 S proteins can bind relatively high amounts of 23 S RNA. The specificity factor which is the ratio between the amounts of homologous and heterologous RNA associated with the same samples of protein is for total 30 S protein as low as 2.

In addition, complex formation between ribosomal RNA's and individual proteins of 30 S subunits has been studied. Proteins of S7 and S20 were chosen. Both proteins can bind 16 S RNA independently of other proteins. But it is only protein S7 that is capable of recognizing both the 16 S and 23 S RNA, as was recently demonstrated by Kurland et al. [8]. It was found (table 2) that the specificity factor for protein S7 is 3, and 10 for S20. We suggest, therefore, that the Sepharose-bound ribosomal proteins studied have the same specificity of association with RNA as in the free state.

The results reported above may be taken to mean that at least in some cases Sepharose-bound ribosomal proteins can be used for studying RNA–protein interactions. We also intend to apply them for isolating protein recognition sites of 16 S RNA by affinity chromatography.

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