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New Procedures for the Preparation of Complexes of Ribosomes with Polyuridylic Acid*

D. M. Logan† and M. F. Singer

ABSTRACT: A new method for the isolation of complexes of polyuridylic acid (poly U) with ribosomes is presented. Under certain conditions of salt and pH, free poly U is adsorbed by polyacrylamide gels while ribosome-bound poly U is not. Using columns of polyacrylamide, complexes of poly U with ribosomes can be separated from free poly U in a matter of minutes. Evidence which indicates that this column technique and the customary sucrose gradient procedure yield similar complexes is presented.

The interaction of poly U[1] with ribosomes has been investigated extensively (Logan and Whitmore, 1966; Moore, 1966a,b; for a review of earlier work see Singer and Leder, 1966). Studies of the properties of the resulting complex are, however, hampered by several factors. The customary method for separating free poly U from ribosome-bound poly U, namely, sucrose density gradient centrifugation, is usually more time consuming than is desirable and the isolated ribosome–poly U complex is contaminated with large amounts of sucrose.

In the course of studying poly U–ribosome complexes as substrates for *Escherichia coli* RNase II (Spahr, 1964; Singer and Tolbert, 1965) we have developed a rapid column technique by which free poly U and ribosome-bound poly U can be separated in a matter of minutes. The method is based on the fact that under certain conditions of pH and salt, free poly U is adsorbed to polyacrylamide gels while poly U bound to ribosomes is not. The action of RNase II on poly U–ribosome complexes obtained by these procedures will be considered in a subsequent publication.

Experimental Procedure

Materials. Copolymers of acrylamide and methylenebisacrylamide (Bio-Gels) were obtained from Bio-Rad Laboratories, Richmond, Calif., and are designated Bio-Gel P-30 and P-100. Blue Dextran 2000 was obtained from Pharmacia, Uppsala, Sweden. [^{14}C]- and [^{3}H]phenylalanines were purchased from the New England Nuclear Corp.

E. coli strain MRE-600 was the kind gift of Dr. H. E. Wade. This mutant is devoid of RNase I (Spahr and Hollingworth, 1961; Cammack and Wade, 1965;

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[1] Abbreviations used: A, U, G, C, and I represent the residues of adenylic, uridylic, guanylic, cytidylic, and inosinic acids, respectively, in polyribonucleotide chains. For example, poly A is polyadenylic acid, and poly UC is a copolymer of uridylic and cytidylic acids. Similarly, poly-4-*N*-methyl C represents poly-N^4-methylcytidylic acid; poly-6-*N*-methyl A, poly-N^6-methyladenylic acid; and poly-6,6-*N*-dimethyl A, poly-N^6,N^6-dimethyladenylic acid. 5′-UMP, uridine 5′-monophosphate; GTP, guanosine triphosphate.

Gesteland, 1966; Singer and Tolbert, 1965). Cells were grown and collected as previously described (Singer, 1966) and were stored frozen. Crude ribosomes were prepared from the cell paste by the method of Nirenberg (1963), omitting the preincubation step.

Poly U, poly C, poly A, poly UG, poly UC, and poly UI were prepared in this laboratory using polynucleotide phosphorylase purified from *Micrococcus lysodeikticus* (Singer, 1966). Poly G was the gift of Dr. Leon A. Heppel (Heppel, 1963). Poly-4-*N*-methyl C (Brimacombe and Reese, 1966), poly-6-*N*-methyl A, and poly-6,6-*N*-dimethyl A (Griffin *et al.*, 1964) were the generous gift of Dr. Colin Reese. $(Up)_6U$ was obtained from the Miles Chemical Co. [^{14}C]- or [^{3}H]-phenylalanyl-tRNA was prepared as described by Pestka (1966) from tRNA isolated from *E. coli* B (Zubay, 1962). A 100,000*g* supernatant fraction from *Lactobacillus arabinosus* served as a source of nuclease-free transfer factors needed for polyphenylalanine synthesis (Salas *et al.*, 1965).

Analytical Procedures. RNase II activity was assayed by the method of Singer and Tolbert (1965). Protein was determined by the procedure of Lowry *et al.* (1951). RNA phosphorus was determined by the method of Chen *et al.* (1956) as modified by Ames and Dubin (1960).

Polyacrylamide Gel Preparation. Gels were prepared by swelling overnight at 4° in the buffer to be used for elution. Hydrated gels were replaced by freshly swelled gels after a maximum of 4–5 days to avoid aging effects (see Results).

Incorporation of Phenylalanine into Polypeptide. Reaction mixtures (0.25 ml) contained: 0.06 M Tris-HCl (pH 7.5), 0.16 M NH_4Cl, 10 mM $MgCl_2$, 1 mM GTP, 12 mM β-mercaptoethanol, 15 μg of poly U, 2.4 A_{260} units of [^{14}C]- or [^{3}H]phenylalanyl-tRNA (containing approximately 12,000 cpm, with from 100 to 150 cpm/$\mu\mu$mole of phenylalanine), between 0.1 and 0.2 mg of the preparation of transfer factors from *L. arabinosus*, and from 1 to 30 μg of ribosomes. After incubation at 37° for 15 min, 0.15 ml of 1 N NaOH was added and the mixture was heated at 100° for 5 min. The solutions were cooled in ice and 0.15 ml of 1 N HCl followed by 3 ml of cold 10% trichloroacetic acid were added. The mixture was filtered through a Millipore filter (pore size, 0.45 μ) and washed three to five times with 5 ml of cold 5% trichloroacetic acid and the filter was counted in 10 ml of scintillation fluid (Bray, 1960) in a Packard Tri-Carb liquid scintillation counter. Under these conditions, incorporation of phenylalanine into acid-insoluble, alkali-stable material requires the presence of poly U, the *L. arabinosus* fraction, and ribosomes. The extent of incorporation is dependent on the concentration of ribosomes between approximately 3 and 20 μg of ribosomes/assay (Figure 1).

Ribosome Purification. The procedure used for the purification of crude ribosomes was designed to minimize the contamination of the particles by ribonuclease. The use of *E. coli* strain MRE 600 as a source of ribosomes essentially eliminates RNase I contamination. In order to remove the RNase II activity generally associated with crude ribosomes (Spahr, 1964; Singer and Tolbert, 1964, 1965) we developed a preparative method, based on Spahr's (1964) observation that ribosomes may be separated from bound RNase II by sedimentation through 5–20% sucrose gradients. The RNase II activity remains close to the top of the sucrose solution while the ribosomes sediment toward the bottom. It is of some interest that no clear separation of RNase II is obtained if the ribosomes are simply sedimented through a 10% sucrose solution. For preparative work we have used 10–30% linear sucrose gradients and have carried out the centrifugation in a fixed angle rotor. The centrifuge is run long enough to allow the ribosomes to form a pellet and the sucrose solution containing the bulk of the RNase II is removed by decantation.

Crude ribosomal preparations were suspended in 0.01 M Tris-HCl (pH 7.5) containing 0.01 M $MgCl_2$. After centrifugation for 2 hr at 100,000*g* the pellet was resuspended with a glass rod in the same buffer and the resulting suspension was clarified at 10,000*g* (10 min). Approximately the top four-fifths of the solution was collected (one-time-washed ribosomes). In some preparations the supernatant was subjected to a second cycle of high- and low-speed centrifugations (two-times-washed ribosomes). The final solution was divided into aliquots and stored at −70°. To purify these ribosomes further, 20–30 A_{260} units of washed ribosomes (~0.1 ml) was layered on top of a 10–30% linear sucrose gradient (volume, 27 ml) containing 0.02 M Tris-HCl (pH 7.5) and 0.005 M $MgCl_2$ in polycarbonate tubes of a Spinco No. 30 rotor. The gradients were centrifuged overnight (12–16 hr) at 0° at 30,000 rpm and the fluid in the tubes was then poured off. The pellet was rinsed lightly with the same buffer to reduce the sucrose concentration and resuspended gently with a glass rod in the same buffer. This suspension was centrifuged for 10 min at 10,000*g* and the top four-fifths was removed, divided into aliquots, and frozen at −70°. Ribosomes prepared in this way are called "sucrose ribosomes." One A_{260} unit of ribosomes is assumed to be equivalent to 69.4 μg dry weight of ribosomes (Nishizuka and Lipmann, 1966) or 24 $\mu\mu$moles of ribosomes (Tissières *et al.*, 1959).

The sucrose gradients may be carried out under conditions other than the one described above. A large number of Mg^{2+} and NH_4^+ concentrations have been tested and found to be suitable. Increasing the quantity of ribosomes applied to each gradient over that described above increases both the recovery of ribosomal material (measured by absorbance) and the specific RNase II activity of the isolated ribosomes (Table I). At the inputs normally used (20–30 A_{260} units/27-ml gradient) residual RNase II activity is about 1–2% that of one-time-washed ribosomes (Table I). Hardy and Kurland (1966) have noted that sucrose gradient centrifugation partially removes bound polynucleotide phosphorylase from ribosomes. Sucrose ribosomes prepared by the procedures described in this report have 0.005 phosphorolysis unit (Singer and Guss, 1962) per A_{260} unit, compared to 0.013 for washed ribosomes.

The data in Figure 1 show that sucrose ribosomes are approximately four times more efficient than one-time-

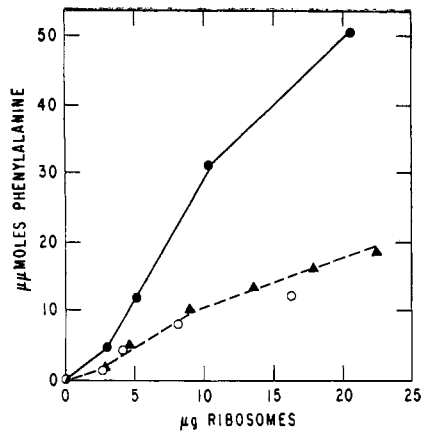

FIGURE 1: Incorporation of [^{14}C]phenylalanine as a function of ribosome concentration. (○, ▲) One-time-washed ribosomes (the two symbols give data obtained on different days); (●) sucrose ribosomes. Assays were carried out as described in the section on methods.

TABLE II: The Binding of Poly U by Sucrose and Crude Ribosomes.[a]

Ribosomes		Poly U	% Poly U
Type	A_{260}	A_{260}	Bound
Crude	7.4	0.46	10
Sucrose	7.4	0.46	41

[a] Ribosomes and [^{14}C]poly U (17,800 cpm) were combined in the stated amounts in a total of 0.5 ml of solution containing 10 mM Tris-HCl (pH 7.5)–10 mM $MgCl_2$. The mixtures were incubated for 10 min at 0°, layered on top of 28 ml of a sucrose gradient (10–30%) in 20 mM Tris (pH 7.5)–5 mM $MgCl_2$, and centrifuged at 24,000 rpm for 5.25 hr in the SW-25 rotor. Fractions (20) were collected by puncturing the bottoms of the tubes, the fractions were counted, and the per cent binding was calculated as indicated in the Methods section.

washed ribosomes in stimulating the poly U directed incorporation of phenylalanine into polypeptide. Various preparations of sucrose ribosomes were consistently between 2.5 and 4 times more efficient than the washed ribosomes. The incorporation reaction, using sucrose ribosomes, is completely dependent on the addition of a source of transfer factors. The sucrose ribosomes can also bind poly U more efficiently than can washed ribosomes (Table II). In the experiments described in Table II, binding was estimated by the sucrose density gradient technique.

The per cent of RNA in one-time-washed and sucrose ribosomes is shown in Table III. The values are somewhat lower than previously published values (Tissières *et al.*, 1959; Furano, 1966) obtained by estimation of RNA content with the orcinol procedure. In our experiments RNA content was estimated from the total phosphorus content. The orcinol procedure could not be used because of the sucrose contaminating the purified ribosomes.

Ribosomes prepared by the sucrose gradient technique in 10 mM Mg^{2+} are less stable than washed ribosomes even when stored at −70°. After 4 weeks the sucrose

TABLE I: Recovery of Ribosomes and RNase II Activity after Sedimentation through Sucrose Gradient.[a]

	Input of Ribosomes	Recov of Sucrose Ribosomes		RNase II
Expt	A_{260}	A_{260}	%	Act.[b]
1	28.0	14.0	50	2.0
	55.0	41.5	76	3.4
	83.0	64.4	78	5.6
	139.0	107	77	8.6
2	24.0			2.2

[a] Samples of crude ribosomes (expt 1) or one-time-washed ribosomes (expt 2) containing the indicated A_{260} units were treated exactly as described for the preparation of sucrose ribosomes in the section on methods. [b] RNase II activity is expressed as micromicromoles of poly A hydrolyzed per minute per microgram of ribosomes in the standard assay (Singer and Tolbert, 1965). The specific RNase II activity of the initial crude ribosomes (expt 1) was greater than 122 μμmoles/min per μg, but an exact measure is not available. The one-time-washed ribosomes used in expt 2 had a specific RNase II activity of 175 μμmoles/min per μg.

TABLE III: RNA and Protein Content of Ribosomes.

Expt	Ribosomes	mg of Protein/ml	mg of RNA/ml[a]	% RNA
1	Washed	11.0	12.9	54
	Sucrose	0.83	1.13	58
2	Washed	16.1	18.4	53
	Sucrose	0.78	0.92	54

[a] The milligrams of RNA per milliliter was calculated from the micrograms of P per milliliter determined as indicated in the Methods section. The per cent P in *E. coli* rRNA was taken as 9.5%; this number is derived from the published base ratios for *E. coli* rRNA (Spahr and Tissières, 1959) and the molecular weights of the mononucleotide residues (the molecular weights of the corresponding nucleoside monophosphates less the weight of 1 equiv of H_2O).

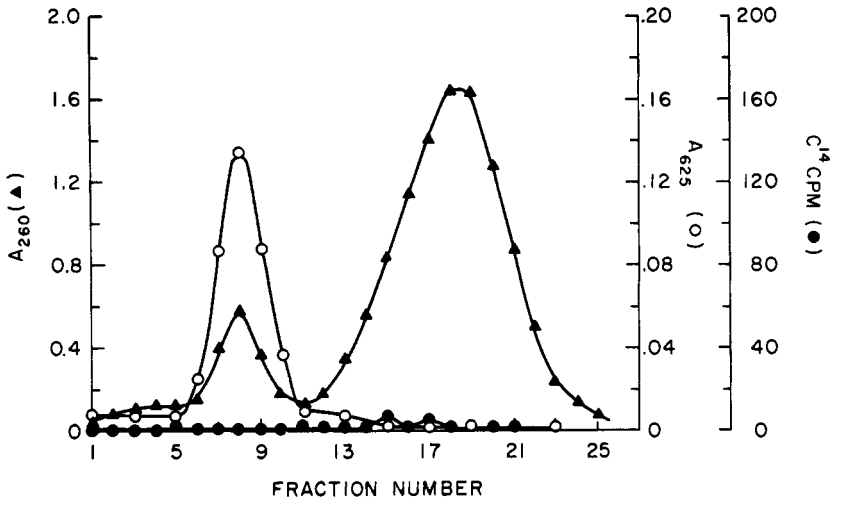

FIGURE 2: The binding of poly U to polyacrylamide. Columns of Bio-Gel P-30 were prepared as described in the section on methods. In one experiment, Blue Dextran 2000 and 13.5 A_{260} units of 5′-UMP were mixed in a final volume of 0.2 ml of 0.02 M Tris-HCl (pH 7.5) containing 5 mM Mg^{2+} and applied to a column (0.9 × 20.6 cm, 13.2 ml) which was eluted with the same buffer. Fractions of 1 ml were collected and their absorbancy was determined at 625 (Blue Dextran) and 260 mμ (Blue Dextran and 5′-UMP). The peak of absorbancy at 260 mμ coinciding with the 625-mμ peak results from the absorbancy of Blue Dextran at that wavelength (in 0.02 M Tris, pH 7.5, the ratio of the absorbance of Dextran Blue at 260 mμ to that at 625 mμ is 3.3). In a separate experiment, 0.92 A_{260} unit of [^{14}C]poly U (containing 9950 cpm) in 0.02 M Tris-HCl (pH 7.5)–5 mM Mg^{2+} was applied to a column (1.4 × 9.7 cm, 15 ml) and eluted as described above. The counts per minute in 0.5-ml aliquots of each fraction were determined.

ribosomes lose essentially all of their ability to incorporate phenylalanine; the stability is not improved by adding 6 mM β-mercaptoethanol to the storage medium. After 3-weeks' storage, the sucrose ribosomes are fully active. On the other hand, washed ribosomes retain full activity for at least 2 months.[2]

Binding of Poly U to Ribosomes. Binding of [^{14}C]poly U to ribosomes was assayed both on sucrose gradients (Takanami and Okamoto, 1963) and on Bio-Gel columns (see below). In the latter case all the poly U in the ribosome peak (identified by absorbancy at 260 mμ) was assumed to be bound. In the gradients all poly U found in the region of approximately 25 S and beyond was considered to be bound. The per cent binding was calculated from the amount bound and the original input of radioactivity. No distinction was made between ribosome-bound and subunit-bound poly U.

Results

The Adsorption of Poly U by Copolymers of Acrylamide and Methylenebisacrylamide. The procedure for the isolation of complexes of poly U with ribosomes that is described below is based on the observation that, under certain conditions, Bio-Gel binds poly U quantitatively. This binding is demonstrated by the data in Figure 2 which compare the elution of Blue Dextran 2000, 5′-UMP, and [^{14}C]poly U from a Bio-Gel P-30 column. The Blue Dextran 2000 is completely excluded from the column; the 5′-UMP, which is completely included, is eluted after approximately one column volume of effluent is collected. In contrast, the [^{14}C]poly U is never eluted from the column. Continued elution with several column volumes of effluent failed to remove significant amounts of [^{14}C]poly U. Porosities designated Bio-Gel P-10 to P-200 were tested and found to be equally effective adsorbants. For the studies reported here, P-30 was generally used since it provides a rapid flow rate and readily separated large molecules from small molecules such as mononucleotides.

The effect of various ions on the adsorption of poly U at pH 7.8 is shown in Table IV. In these experiments column volumes of 4–5 ml were used and the input of poly U was either 4.95 or 12.4 A_{260} units. Recovery from the column was assessed by the recovery of A_{260} in the effluent. In 0.01 M Tris–5 mM Mg^{2+}, the adsorption is quantitative but it decreases with decreasing Mg^{2+} and in the presence of EDTA. The binding is essentially eliminated at high concentrations of phosphate and is severely reduced at 0.01 M phosphate (possibly as a result of the complexing of Mg^{2+} by phosphate). In the cases where incomplete binding occurred, some "leakage" was seen and small amounts of poly U were recovered continuously over two to three column volumes of effluent.

Adsorbed poly U can be eluted quantitatively in one

[2] Some of these experiments were carried out by Dr. James J. Castles.

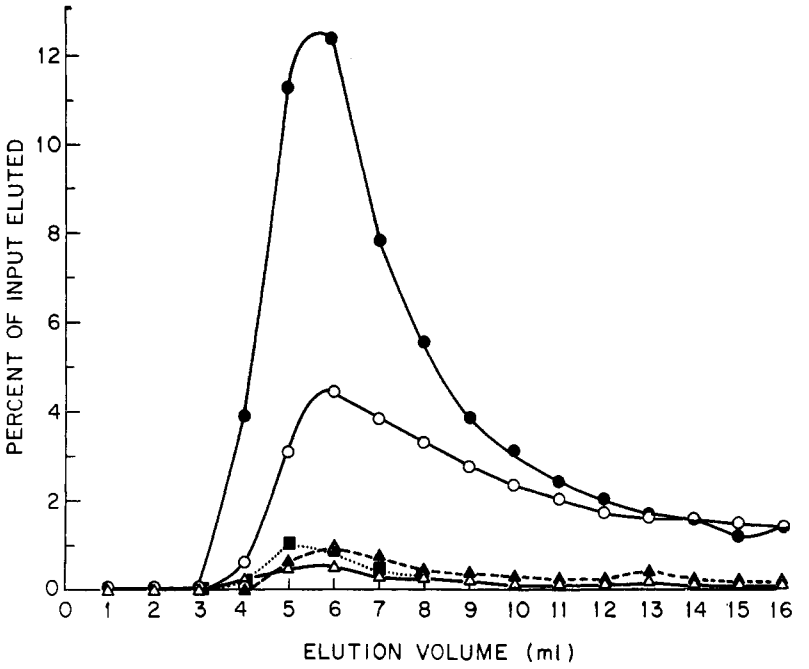

FIGURE 3: The effect of pH on the adsorption of poly U by polyacrylamide gel. All columns (6 cm high) contained 4 ml of freshly swollen P-30 gel equilibrated with one of the buffers indicated below. In each case, the input was 0.54 A_{260} unit of [^{14}C]poly U (12,500 cpm). Sixteen 1-ml fractions were collected from each column and the radioactivity was determined. The following buffers (0.01 M containing 0.005 M $MgCl_2$) were tested (the per cent of the total input radioactivity recovered in two column volumes after the void volume is given in parentheses): sodium cacodylate, pH 5.62 (2.4), 6.30 (3.6), and 7.20 (3.8); and Tris-HCl, pH 7.80 (2.9), 8.1 (3.6), 8.60 (4.4), 8.80 (22.6), and 9.10 (50.0). The elution profiles for pH 9.1 (—●—), 8.8 (—○—), 8.1 (· ·■· ·), 7.2 (- -▲- -), and 5.62 (—△—) are illustrated. Not all the points are plotted at pH 8.1; the remaining fractions all contained less than 0.3% of the input counts.

column volume with 1 M KCl (in Tris-HCl buffer). At lower KCl concentration (0.2 M) the recovery is complete but requires about three column volumes of effluent.

The effect of pH on the binding was investigated using 0.01 M sodium cacodylate or 0.01 M Tris-HCl buffers containing 0.005 M $MgCl_2$ (Figure 3). In the pH range 5.6–8.6 essentially all the poly U is bound to the column. Above pH 8.6 the recovery of poly U increases strikingly. Many experiments performed between pH 7.8 and 8.2 have consistently shown that between 97 and 100% of the input poly U is adsorbed.

TABLE IV: Effect of Mg^{2+} on Binding of Poly U to Polyacrylamide Gel (P-30, pH 7.8).[a]

Expt	Medium	% Adsorbed
1	Tris (0.01 M)	84
2	Tris (0.01 M)–Mg^{2+} (1 mM)	>91
3	Tris (0.01 M)–Mg^{2+} (5 mM)	100
4	Tris (0.01 M)–EDTA (10 mM)	60
5	Phosphate (0.1 M)–Mg^{2+} (5 mM)	5
6	Phosphate (0.01 M)–Mg^{2+} (5 mM)	45

[a] Column volumes were 4–5 ml; five column volumes of effluent were collected. In expt 1, 2, and 5 the input was 12.4 A_{260} units of poly U. In the other experiments the input was 4.95 A_{260} units of poly U.

Capacity and Aging of Gels. The ability of gels to adsorb poly U quantitatively decreases with age when they are stored in buffer, although the shelf life of dry gel appears to be indefinite. Gels which adsorb 97–100% of the input poly U when fresh will adsorb only about 85–90% after 10 days of storage at 4°. Similarly, previously adsorbed poly U will start to leak off columns after 2-weeks' storage at 4°. Within this limitation, the capacity of the gel is extremely high. With inputs of 26, 76, and 161 A_{260} units of poly U/g of dry gel, the recovery at pH 7.8 is 0–3, 5, and 8%, respectively, for freshly prepared gel. In each case a minimum of three column volumes of effluent was collected.

Binding of Other Polynucleotides. A variety of other polyribonucleotides was tested for binding on the columns and the results are shown in Table V. With the exception of poly G which has anomalous behavior in other chromatography systems (Lipsett and Heppel, 1963), the only polynucleotides tested which showed significant binding at pH 7.8 were UC copolymers having high proportions of U. UC copolymers with U contents

TABLE V: Adsorption of Polyribonucleotides to Polyacrylamide Gels (P-30).[a]

Polymer	Input	Column Size (ml)	% Eluted
Poly C	17.2 A_{269}	6.5	91
Poly G	5.7 A_{252}	6.5	13
Poly-4-*N*-methyl C	6.6 A_{270}	4.6	86
Poly-6-*N*-methyl A	5.1 A_{257}	4.6	86
Poly-6,6-*N*-dimethyl A	2.5 A_{257}	5.0	98
Poly UC (70:30)	3.8 A_{257}	5.0	49
Poly UC (77:23)	6.9 A_{260}	5.0	1.8
Poly UC (30:70)	5.9 A_{260}	5.0	90
Poly A	4.9 A_{260}	5.0	99
$(Up)_6U$	2.2 A_{260}	4.0	96

[a] All in 0.01 M Tris (pH 7.8) (except $(Up)_6U$ where 0.02 M was used)–5 mM Mg^{2+} at 4°. Three to five column volumes of effluent collected.

ranging from 30 to 77% were tested for adsorption In the former case only 10% of the input polymer was adsorbed, whereas in the latter more than 98% was retained by the column. A heptanucleotide containing exclusively uridine residues was not adsorbed at all.

Binding of Poly U to Ribosomes. The data in Figure 4 demonstrate that when poly U is mixed with ribosomes, a portion of the poly U is no longer adsorbed to polyacrylamide, but is eluted in a peak coincident with the ribosome peak. Thus, this technique appears to provide a rapid method for assessing the extent of binding of poly U to ribosomes and for isolating poly U–ribosome complexes. Owing to the high capacity of the gel for poly U, columns of small volume are quite satisfactory and provide minimal dilution of the input sample. In the experiment shown in Figure 4, approximately 20% of the poly U was eluted with the ribosomes. In similar experiments the per cent of poly U bound to ribosomes as measured by the column procedure was compared with the per cent bound as measured by the more customary sucrose gradient technique. The input ratio of poly U to ribosomes was varied, and typical results are given in Table VI. Both crude and sucrose ribosomes were tested. The experiments with crude ribosomes show that the per cent of poly U bound increased when the input ratio of poly U to ribosomes was decreased. This observation confirms earlier experiments (Takanami and Okamoto, 1963) and further supports the notion that the column technique is indeed measuring binding. At all input ratios, and with both crude and sucrose ribosomes, the binding assessed by the column technique exceeds that measured by sucrose gradient centrifugation by between 50 and 100%. Therefore, to establish more firmly that the column technique and the gradient technique are indeed measuring the same thing, the poly U–ribosome complex recovered from a Bio-Gel P-30 column was centrifuged on a 5–20% sucrose gradient. A mixture of 3.8 A_{260} units of sucrose ribosomes and 0.92 A_{260} unit of poly U were preincubated for 5 min, at 4°, in a total volume of 0.21 ml of 0.01 M Tris-HCl (pH 7.5)–10 mM Mg^{2+} and then applied to a 5-ml column of Bio-Gel P-30 equilibrated in 0.01 M Tris-HCl (pH 7.8)–5 mM Mg^{2+}. Fractions of 1 ml were collected, and small aliquots were counted. As expected, a peak of radioactivity coincided with the void volume of the column. The peak fraction was layered on top of a 5–20% sucrose gradient prepared in the same buffer as above and centrifuged for 3 hr at 24,000 rpm in the SW-25 head. An absorbancy profile of the gradient was obtained and 29 1-ml fractions were collected and counted. Of the radioactivity recovered from the gradient, 78% coincided with the ribosomes on the gradient and 22% remained near the top of the tube. This loss is consistent with the discrepancies in per cent binding noted in Table VI.

The binding of poly U to *E. coli* ribosomes, as measured by sucrose gradients, is known to be dependent on Mg^{2+} concentration. It was of some interest therefore to study the magnesium dependence of binding measured by the column technique. The results of such a study are given in Table VII. Binding is a function of Mg^{2+} concentration, and the optimal binding is seen at 0.02 M Mg^{2+}.

Discussion

The selective adsorption of poly U by the copolymers of acrylamide and methylenebisacrylamide (Bio-Gels) provides a rapid and simple procedure for the isolation of complexes of poly U with ribosomes. The chemical basis for the adsorption is not known. It is clear that divalent cation (Mg^{2+}) is required and high concentrations of KCl eliminate binding. As indicated in the legend to Table VII, poly U is efficiently bound at Mg^{2+} concentrations up to 0.03 M. Under the conditions reported in this paper, only poly U or polymers containing a high per cent of U residues are bound. The experiment with $(Up)_6U$ suggests that adsorption depends on chain length. This conclusion is supported by unpublished data showing that a heterogeneous poly U of average chain length 20 is only partly adsorbed to Bio-Gel.We have also noted that poly A has some tendency to bind to Bio-Gel at pH values less than 6. Poly U also binds to methylated albumin columns (Asano, 1965).

The formation of poly U–ribosome complexes has generally been studied by means of sucrose density gradient centrifugation. Detection of complex formation has also been carried out by a technique involving the use of Millipore filters (Moore, 1966a). The method described in this report permits the rapid isolation of the complexes and has the additional advantage that the resulting material is free of sucrose. Several lines of evidence support the notion that the Bio-Gel method and the sucrose gradient technique do indeed yield similar complexes. First, the ratio of poly U bound by

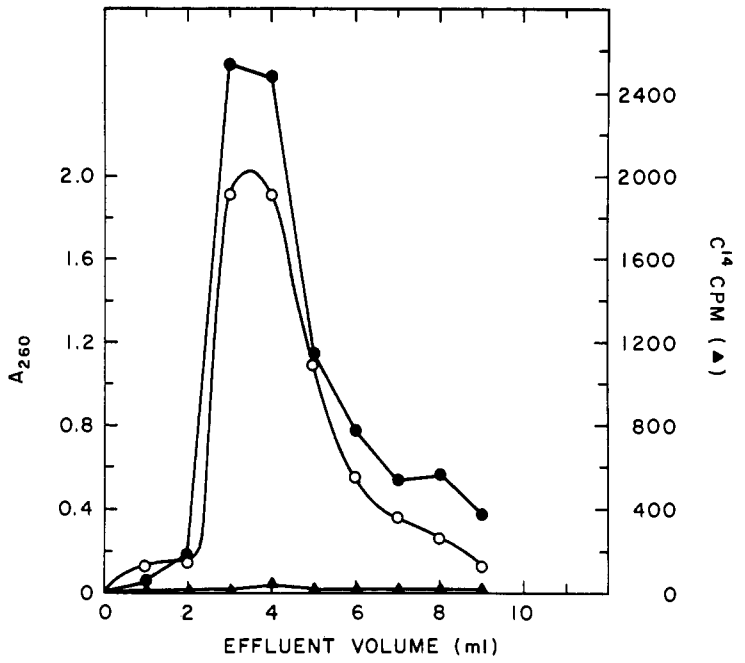

FIGURE 4: The elution of poly U–ribosome complex from Bio-Gel P-200. A column (0.9 × 5.7 cm) of Bio-Gel P-200 was prepared as described in the section on methods and equilibrated with 10 mM Tris (pH 7.5)–5 mM Mg^{2+}. Sucrose ribosomes (7 A_{260} units) and poly U (0.9 A_{260} unit containing 45,000 cpm) were mixed in a final volume of 0.5 ml containing 10 mM Tris-HCl (pH 7.5), 0.05 M KCl, 5 mM Mg^{2+}, and 1 mM β-mercaptoethanol and the mixture was applied to the column. Elution was with the above buffer. Fractions of 0.95 ml were collected, their absorbance was determined at 260 mμ, and 0.5 ml of each was used for determining radioactivity. (○) A_{260} and (●) counts per minute per fraction. A control mixture lacking ribosomes was also passed through the column and radioactivity was determined as above. The control data are indicated by the symbol ▲.

TABLE VI: Binding of Poly U to Ribosomes. Comparison of Gradient and Column Methods.[a]

Expt	Ribosomes		Poly U		% Poly U
	Type	A_{260}	A_{260}	Method[b]	Bound
1	Crude	12.5	0.26	Column	28
	Crude	12.5	0.26	Gradient	16
2	Crude	12.5	0.05	Column	52
	Crude	12.5	0.05	Gradient	35
3	Sucrose	7.4	0.46	Column	60
	Sucrose	7.4	0.46	Gradient	41

[a] Ribosomes and poly U were mixed in the indicated amounts. After 10-min incubation at 0°, the samples were applied either to sucrose gradients (10–30% in 0.02 M Tris (pH 7.5)–5 mM $MgCl_2$, 27-ml total volume) or to P-30 columns (1.4 × 9.7 cm, equilibrated with 0.02 M Tris (pH 7.5)–5 mM $MgCl_2$). Binding was estimated as indicated in the Methods section and in the legend to Table II. Experiments 1 and 2, ribosomes and poly U were mixed in a solution containing 0.02 M Tris (pH 7.5)–5 mM $MgCl_2$. Experiment 3, the binding mixtures contained 0.02 M Tris (pH 7.5)–10 mM $MgCl_2$. [b] Column, P-30 column; gradient, sucrose gradient 10–30%.

washed (or crude) ribosomes to that bound by sucrose ribosomes is the same when results obtained by the two methods are compared. Second, the two assays yield similar results when the extent of binding is studied as a function of the input ratio of poly U to ribosomes. Third, when measured by the column technique, the extent of binding is a function of the Mg^{2+} concentration; the optimal concentration of Mg^{2+} is 0.02 M.

TABLE VII: Binding of [^{14}C]Poly U to Sucrose Ribosomes. Effect of Magnesium Concentration.[a]

Mg^{2+} (M)	Cpm Bound
0.005	1778
0.010	2156
0.015	3142
0.020	4888
0.024	4398
0.030	4104

[a] Binding was measured on P-100 columns (5 ml) as outlined in the Methods section. The buffer in each case was 0.01 M Tris (pH 7.5)–$MgCl_2$ of the indicated concentration. The input in each case was 5.3 A_{260} units (250 μl) of sucrose ribosomes and 0.78 A_{260} unit (30 μl) of [^{14}C]poly U (9300 cpm total) in the same buffer as the test column. Control columns run in the absence of ribosomes indicated that poly U is bound to the P-100 at all Mg^{2+} concentrations tested.

These results mirror those obtained by earlier workers using the sucrose gradient procedure (Spyrides and Lipmann, 1962; Takanami and Okamoto, 1963) and a Millipore filter method (Moore, 1966a). Finally, 80% of the poly U in a poly U–ribosome complex isolated by the column procedure sediments as complex when subjected to gradient centrifugation. The loss of approximately 20% of the poly U is consistent with the observation that the extent of binding is always somewhat greater when measured by the column technique than when measured by centrifugation. This discrepancy may reflect the influence of strong physical forces on the complex. One disadvantage of the column procedure is the lack of differentiation between polysomes and monosomes.

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