The Effect of Polyamines on the Binding of Aminoacyl Transfer Ribonucleic Acid to Ribosomes in a Yeast System*

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ABSTRACT: The binding of yeast tyrosyl transfer ribonucleic acid to yeast ribosomes with uridylyl-(3′,5′)-adenylyl-(3′,5′)-uridine is weak in the absence of polyamines. Binding is markedly stimulated by spermidine, and a level of binding similar to that observed in the *Escherichia coli* system can be attained. Other polyamines differ widely in their effect on the binding reaction, putrescine inhibiting it. The presence of spermidine lowers the optimal magnesium requirement of the reaction but leaves the other characteristics of the binding reaction unaltered. The site of action of spermidine is the ribosome, but no change in the state of aggregation of the ribosome is observed when the polyamine is present.

Several workers have reported the stimulation of phenylalanine incorporation in yeast cell-free protein synthetic systems by polyuridylic acid (Bretthauer *et al.*, 1963; Heredia and Halvorson, 1966; Dietz *et al.*, 1965; Downey *et al.*, 1965; Söll *et al.*, 1966). The requirements of the yeast system are very similar to those of *Escherichia coli* cell-free protein synthetic systems (Nirenberg and Matthaei, 1961), but amino acid incorporation in the former is stimulated by spermidine and spermine (Bretthauer *et al.*, 1963).

Recently the binding of yeast phenylalanyl-tRNA to washed yeast ribosomes with polyuridylic acid has been reported (Heredia and Halvorson, 1966), as has the trinucleotide-stimulated binding of several yeast aminoacyl-tRNA species to yeast ribosomes (Söll *et al.*, 1966). The results in the latter paper were obtained using yeast ribosomes purified by density gradient centrifugation and the conditions for binding of the *E. coli* system (Nirenberg and Leder, 1964), but the stimulations of binding to yeast ribosomes which were obtained were generally poorer than those obtained with the *E. coli* system (Söll *et al.*, 1965; Brimacombe *et al.*, 1965). This paper describes a cell-free-binding system from a brewer's yeast in which the trinucleotide-stimulated binding of aminoacyl-tRNA to ribosomes is of a similar order of magnitude as that obtained with the *E. coli* system, when certain polyamines are present.

Materials and Methods

Brewer's yeast was the gift of the Star Brewery, Cambridge. UAC[1] was prepared by the method of Thach and Doty (1965). UAU was a gift from Dr. B. E. Griffin and *E. coli* tRNA and *E. coli* ribosomes were gifts from Dr. B. F. C. Clark.

Growth of Yeast. Yeast was grown in a synthetic medium adapted from that of Williams and Dawson (1952). An inoculum of the yeast was grown up to stationary state in a 100-ml culture overnight at 30°. This culture was used as an inoculum for a 10-l. culture grown at 30° with vigorous agitation and maximum aeration. When the $OD_{650\ m\mu}$ reached 2.0 the culture was poured onto ice chips and the cells were immediately centrifuged. The cells were washed three times in the centrifuge with 250 ml of cold deionized water. The yield of packed cells from a 10-l. culture was 25–30 g.

Preparation of Yeast Ribosomes, S-30, and Activating Enzyme. The procedure is based on a method used for extracting *E. coli* ribosomes (Clark and Marcker, 1966). Fresh unfrozen yeast cells were always used, and the complete preparation was carried out at 4° and in a single day.

The cells were washed in the centrifuge with extracting buffer, which contained 0.01 M Tris-Cl (pH 7.5), 0.005 M $Mg(OAc)_2$, 0.01 M KCl, 0.01 M β-mercaptoethanol, and 0.25 M sucrose. Potassium cacodylate buffer (0.1 M, pH 7.2) was substituted for Tris in this buffer when the cell-free extract was to be used for protein synthesis experiments.

Cell disruption was accomplished by grinding the cells either with three times their weight of acid-washed sand or with twice their weight of levigated alumina. Alumina grinding gave a better yield of ribosomes and these ribosomes were more active for

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[1] Abbreviations used: UAC, uridylyl-(3′,5′)-adenylyl-(3′,5′)-cytidine; PEP, phosphoenolpyruvate; ATP, adenosine 5′-triphosphate; GTP, guanosine 5′-triphosphate; UAU, uridylyl-(3′,5′)-adenylyl-(3′,5′)-uridine; OD_{260} unit, the quantity of material contained in 1 ml of a solution which has an optical density of 1 at 260 mμ when measured in a 1-cm path-length cell.

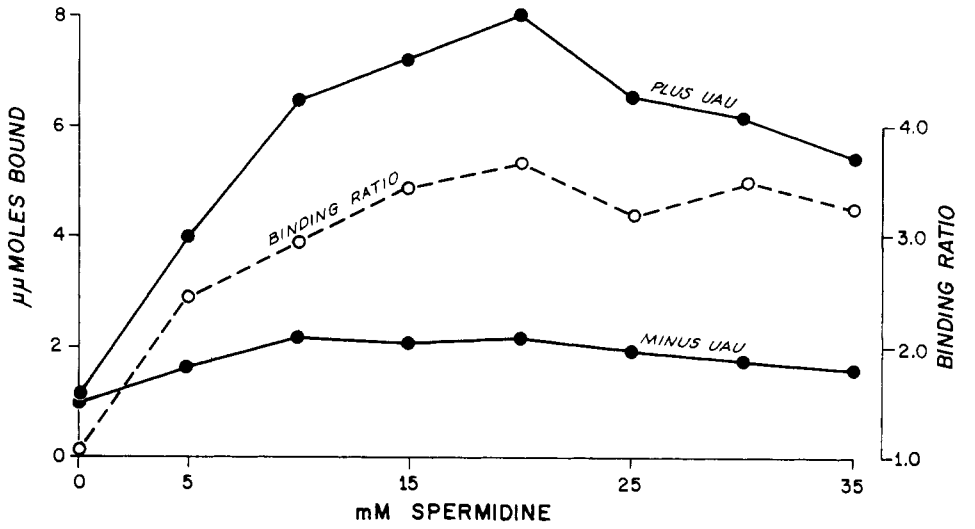

FIGURE 1: Effect of spermidine on binding of yeast [^{14}C]tyrosyl-tRNA to yeast ribosomes. Binding was carried out as indicated in Materials and Methods. Spermidine was the only polyamine present.

protein synthesist han ribosomes obtained from sand-ground cells. Both methods yielded ribosomes which were active in the binding assay.

Cells (30 g) were ground with the abrasive in a mortar and pestle precooled to −20°. After grinding for about 10 min the paste was extracted with 60 ml of extraction buffer which also contained 3 μg/ml of DNase, 1 mM spermidine, and 0.02 mM spermine. The mixture was centrifuged at 10,000*g* for 10 min, and the supernatant was recentrifuged at 20,000*g* for 30 min. This supernatant was then centrifuged at 30,000*g* for 30 min, the pellet was discarded, 10 μl/ml of 0.05 M PEP, 5 μl/ml of 0.1 M ATP, and 4 μg/ml of pyruvate kinase were added to the supernatant, and the mixture was preincubated at 30° for 30 min. This extract was designated the S-30, and for protein synthesis experiments small aliquots of this solution were stored under liquid nitrogen.

To obtain washed ribosomes, the S-30 was centrifuged at 150,000*g* for 120 min. The supernatant, after dialysis against a solution containing 0.01 M Tris (pH 7.5), 0.01 M $MgCl_2$, and 0.01 M β-mercaptoethanol, was used as activating enzyme for the charging of the tRNA with radioactive amino acids.

The pellets from the 150,000*g* centrifugation were rinsed with extracting buffer and homogenized with 40 ml of extraction buffer. After adding 2 ml of 1 M Tris-Cl (pH 7.4) and 0.8 ml of 5% sodium deoxycholate to the homogenate, the solution was gently mixed and centrifuged at 150,000*g* for 120 min. The pellets were rinsed and homogenized in 40 ml of extraction buffer, the homogenate was centrifuged at 10,000*g* for 10 min to remove aggregates, and the supernatant was recentrifuged at 150,000*g* for 120 min. The pellet surfaces were rinsed and the pellets were homogenized in 5 ml of extraction buffer. After a final centrifugation at 10,000*g* for 10 min, the washed ribosome suspension, of OD_{260} equal to 300–500, was frozen in small aliquots under liquid nitrogen. When stored in this way, the ribosomes were active for 2–3 months in the binding assay.

Binding Ratio. The binding ratio is defined as the ratio of the micromicromoles of aminoacyl-tRNA bound in the presence of messenger to the micromicromoles of aminoacyl-tRNA bound in the absence of messenger. When the characteristics of the binding process are studied, the position of maximal binding, expressed as the micromicromoles of aminoacyl-tRNA bound in the presence of messenger, is usually the same as the position of the maximal binding ratio. In certain cases (for example, see Figure 2) the nonspecific binding observed in the absence of messenger is variable and depends on the characteristic which is under study. Under these circumstances the optimal binding ratio appears at a different position to that of maximal binding. It is felt that the binding ratio, by taking the nonspecific binding into account, gives a more meaningful description of the specificity of the binding process. For this reason optima derived from the binding ratio are considered to be more significant than those obtained from the position of maximal binding.

Preparation of Yeast tRNA. The yeast tRNA was extracted from the same brewer's yeast as was used for the preparation of the ribosomes. The partial fractionation procedure used to separate the tRNA species will be described in a subsequent paper (D. M. Brown, B. F. C. Clark, M. J. A. Tanner, manuscript in preparation).

Binding of Aminoacyl-tRNA to Ribosomes. Charging of aminoacyl-tRNA with radioactive amino acids was carried out by the procedure of Clark and Marcker (1966). [^{14}C]Tyrosine (225 mc/mmole), [^{14}C]lysine (189 mc/mmole), and [^{14}C]phenylalanine (222 mc/mmole) were used for charging. The specific acceptor activities

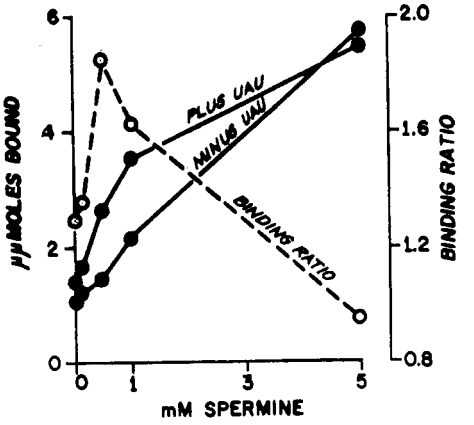

FIGURE 2: Effect of spermine on binding of yeast [^{14}C]-tyrosyl-tRNA to yeast ribosomes. Binding was carried out as indicated in Materials and Methods. Spermine was the only polyamine present.

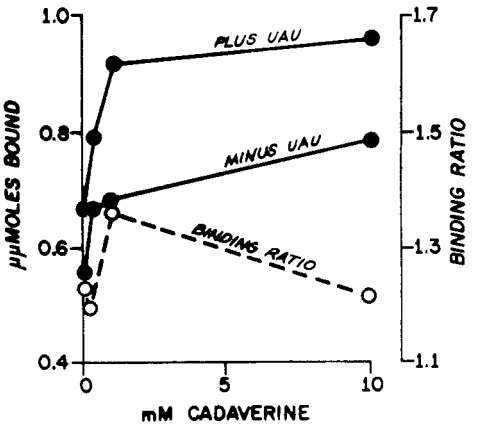

FIGURE 3: Effect of cadaverine on binding of yeast [^{14}C]tyrosyl-tRNA to yeast ribosomes. Binding was carried out as indicated in Materials and Methods. Cadaverine was the only polyamine present.

of the aminoacyl-tRNAs were: tyrosyl-tRNA, 96 μμmoles of tyrosine/OD_{260} unit of tRNA; lysyl-tRNA, 50.5 μμmoles of lysine/OD_{260} unit of tRNA; and phenylalanyl-tRNA, 23 μμmoles of phenylalanine/OD_{260} unit of tRNA.

Binding of aminoacyl-tRNA to ribosomes was assayed by the method of Nirenberg and Leder (1964). The complete reaction mixture for [^{14}C]tyrosyl-tRNA binding to yeast ribosomes contained in a total volume of 50 μl: 10 mM magnesium ions, 50 mM KCl, 100 mM Tris-OAc (pH 7.2), 20 mM spermidine, 0.4 mM spermine, 2.05 OD_{260} units of yeast ribosomes, 32 μμmoles of yeast [^{14}C]tyrosyl-tRNA, and 0.155 OD_{260} unit of UAU. Changes in this basic reaction mixture are noted in individual cases. After incubation at 20° for 20 min the reaction mixtures were kept at 0° prior to membrane filtration.

Results

Effect of Growth Phase on Trinucleotide-Stimulated Binding. The amino acid incorporating activity of yeast cell-free extracts has been shown to vary during the logarithmic phase of growth of the yeast (Lucas *et al.*, 1964) and this variation has been shown to be associated with a change in the yeast ribosome (Dietz *et al.*, 1965).

Ribosomes were extracted from yeast in the stationary state, and at approximately the mid-log phase of growth in a synthetic medium (OD_{650} = 2.0; see Materials and Methods). Ribosomal activity was assayed by determining the binding of yeast [^{14}C]tyrosyl-tRNA to the ribosomes in the presence and absence of UAU. The major binding codons of this tRNA to *E. coli* ribosomes are UAC and UAU (D. M. Brown, B. F. C. Clark, and M. J. A. Tanner, manuscript in preparation). Table I shows the results of this experiment. The stationary-state ribosomes showed slight stimulation of Tyr-tRNA binding with UAC, but their capacity to bind aminoacyl-tRNA was lower than that of log-phase yeast ribosomes. The results suggest that the change in activity of the yeast ribosome first noted by Dietz *et al.* (1965) may take place at the binding step of the protein synthetic reaction.

Effect of Polyamines on Trinucleotide-Stimulated

TABLE I: Effect of Growth Phase on the Activity of Yeast Ribosomes in the Binding Reaction.[a]

	μμmoles of [^{14}C]Tyr-tRNA Bound/OD_{260} Unit of Ribosomes		
	Stationary-State Yeast	Log-Phase Yeast	
Trinucleoside Diphosphate	Washed Ribosomes	Nondeoxycholate-Washed Ribosomes[b]	Washed Ribosomes
None	0.086	0.32	0.38
0.2 OD_{260} unit of UAC	0.104		
0.2 OD_{260} unit of UAU		0.38	0.455

[a] Binding was carried out in the presence of 0.03 M magnesium ions, using 1.5–2.0 OD_{260} units of yeast ribosomes and 32 μμmoles of [^{14}C]Tyr-tRNA. No polyamines were present in the reaction mixture. Extraction of ribosomes by other procedures (Brethauer *et al.*, 1963; Lucas *et al.*, 1964) using several methods of cell disruption gave similar results. [b] The preparation of these ribosomes omitted the sodium deoxycholate washing step.

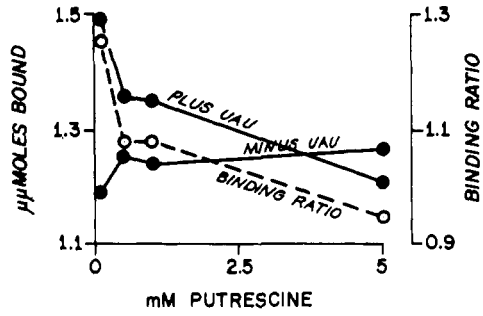

FIGURE 4: Effect of putrescine on binding of yeast [^{14}C]-tyrosyl-tRNA to yeast ribosomes. Binding was carried out as indicated in Materials and Methods. Putrescine was the only polyamine present.

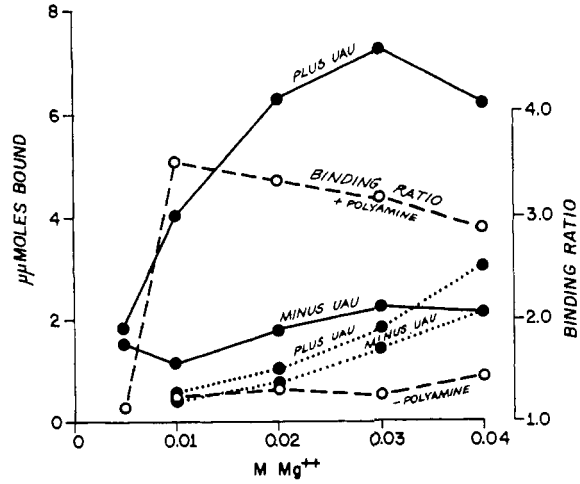

FIGURE 5: Effect of polyamine mixture on the magnesium ion requirements for binding of yeast [^{14}C]tyrosyl-tRNA to yeast ribosomes. (●——●) Micromicromoles bound in the presence of 20 mM spermidine and 0.4 mM spermine; (●· · ·●) micromicromoles bound in the absence of polyamines; (O– – –O) binding ratios. Binding was carried out as described in Materials and Methods. Various concentrations of magnesium ions were used as shown.

Binding. Even with deoxycholate-washed yeast ribosomes, trinucleotide stimulation of binding was slight (binding ratio, 1.2) compared with that obtained in the *E. coli* system. The presence of certain polyamines, particularly spermidine, greatly stimulated the binding reaction. The effect of various concentrations of spermidine on the UAU-stimulated binding of tyrosyl-tRNA is shown in Figure 1. Optimal binding was reached at a concentration of 20 mM spermidine with a binding ratio of 3.6. Both the capacity of the ribosomes to bind tRNA and the binding ratio were similar to the values reported for the *E. coli* system (Nirenberg and Leder, 1964).

Other polyamines showed marked differences in their influence on the binding reaction. Spermine caused some stimulation of binding (Figure 2), the binding ratio reaching an optimum at 0.5 mM spermine. However, in this case the nonspecific binding (the binding observed in the absence of trinucleotide) increased steadily with increasing spermine concentration, so that at high spermine concentrations the nonspecific binding was greater than the binding obtained in the presence of trinucleotide.

Cadaverine (Figure 3) showed a small effect on the binding ratio. Putrescine (Figure 4) inhibited trinucleotide-stimulated binding at all concentrations, the inhibition increasing with putrescine concentration, although the nonspecific binding was little affected by putrescine. The diverse behavior of these polyamines may reflect their differing abilities to complex with RNA.

Spermidine and spermine show their greatest stimulation of the binding reaction at polyamine concentrations in the molar ratio of approximately 50:1. The effect of 50:1 mixtures of these polyamines was tested to determine whether the polyamines would cooperatively stimulate binding. Slight cooperative stimulation by the two polyamines did occur, resulting in increased binding of tyrosyl-tRNA and an increased binding ratio. The results were very similar to those obtained when spermidine alone was employed (Figure 1), the optimal binding ratio being achieved with a mixture of 20 mM spermidine and 0.4 mM spermine. Table II shows the effect on the binding reaction of 20 mM spermidine and 0.4 mM spermine separately, and mixed together, in the same assay set. In the remaining experiments to be described, this mixture of polyamines was used routinely.

The presence of the polyamine mixture lowered the magnesium ion concentration required for the optimal binding ratio from 0.04 M or a higher magnesium ion concentration to 0.01 M magnesium ions, but did not replace it (Figure 5). This result and the evidence that the polyamine stimulation of binding is relatively specific to spermidine suggest that the polyamine is not merely replacing the ribosomal requirement for

TABLE II: Effect of Polyamine Mixture on Binding to Yeast Ribosomes.[a]

Polyamine	μμmoles of [^{14}C]-Tyr-tRNA Bound		Binding Ratio
	Minus UAU	Plus UAU	
20 mM spermidine	2.11	6.86	3.27
0.4 mM spermine	1.48	2.49	1.68
20 mM spermidine plus 0.4 mM spermine	2.46	8.90	3.62

[a] Binding was carried out as described in Materials and Methods, except where otherwise indicated.

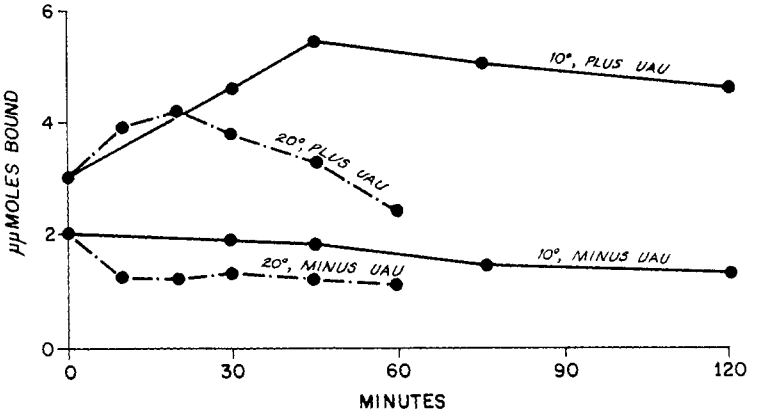

FIGURE 6: Kinetics of binding of yeast [^{14}C]tyrosyl-tRNA to yeast ribosomes in the presence of the polyamine mixture. Binding was carried out as indicated in Materials and Methods. Incubation was carried out at 10 and 20° for the time shown.

cations, but that it also has a unique function in the activity of the yeast ribosome in the binding reaction.

When the polyamines are present the characteristics of the yeast binding reaction are similar to the characteristics of the binding reaction in the *E. coli* cell-free system (Nirenberg and Leder, 1964). The kinetics of binding at 10 and 20° in the presence of 0.01 M magnesium ions are shown in Figure 6. Maximal binding and the optimal binding ratio were attained after 20 min at 20° and after 75 min at 10°. The system also showed the characteristic saturation of tyrosyl-tRNA binding as increasing amounts of UAU were added.

Figure 7 shows the amount of tyrosyl-tRNA bound to ribosomes in the presence of UAU when various amounts of tyrosyl-tRNA were added to the system. The ribosomes became saturated with tRNA when 5 μμmoles of aminoacyl-tRNA was bound to 1 OD_{260} unit of ribosomes. Assuming a molecular weight of 4.5×10^6 for the yeast 80S ribosome (Petermann, 1964) and that 1 OD_{260} unit of washed yeast ribosomes is equivalent to 0.106 mg of ribosomes (Dietz *et al.*, 1965), the system reached saturation with tRNA when 20% of the ribosomes carried one aminoacyl-tRNA. When small amounts of tRNA were used 30% of the tRNA present in the reaction mixture became bound to ribosomes.

Effect of Polyamines on Polynucleotide-Stimulated Binding. The effect of the polyamines on the poly A stimulated binding of yeast lysyl-tRNA (Figure 8) was similar to their effect on the UAU-stimulated binding of tyrosyl-tRNA. Binding was slight in the absence of polyamine and moderate when polyamine was present (optimal binding ratio, 4.3). The optimal binding ratio was observed when 5 mM spermidine and 0.1 mM spermine were present in the reaction mixture.

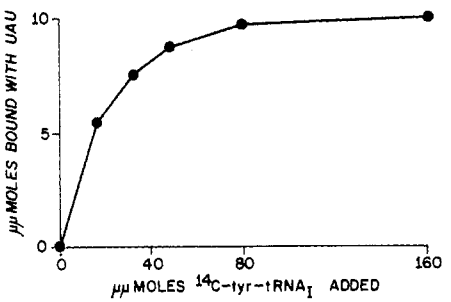

FIGURE 7: Effect of [^{14}C]tyrosyl-tRNA concentration on binding of [^{14}C]tyrosyl-tRNA to yeast ribosomes. Binding was carried out as shown in Materials and Methods, various amounts of [^{14}C]tyrosyl-tRNA being added as indicated.

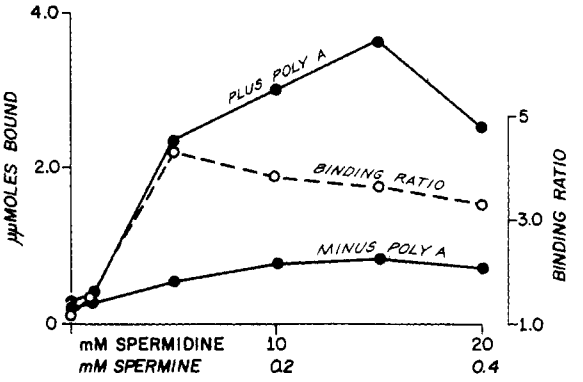

FIGURE 8: Effect of polyamine mixture on poly A stimulated binding of [^{14}C]lysyl-tRNA to yeast ribosomes. Binding was carried out as indicated in Materials and Methods for [^{14}C]tyrosyl-tRNA binding, except that 0.355 OD_{260} unit of poly A and 26.6 μμmoles of [^{14}C]-lysyl-tRNA were used in each assay instead of [^{14}C]-tyrosyl-tRNA and UAU.

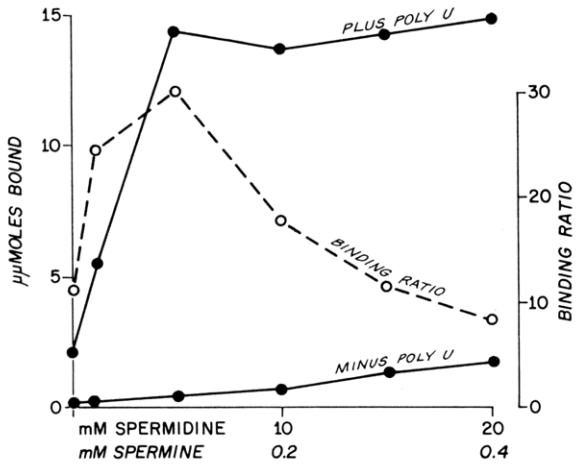

FIGURE 9: Effect of polyamine mixture on poly U stimulated binding of [^{14}C]phenylalanyl-tRNA to yeast ribosomes. Binding was carried out as indicated in Materials and Methods for [^{14}C]tyrosyl-tRNA binding, except that 0.7 OD_{260} unit of poly U and 40.5 $\mu\mu$moles of yeast [^{14}C]phenylalanyl-tRNA were used in each assay instead of [^{14}C]tyrosyl-tRNA and UAU.

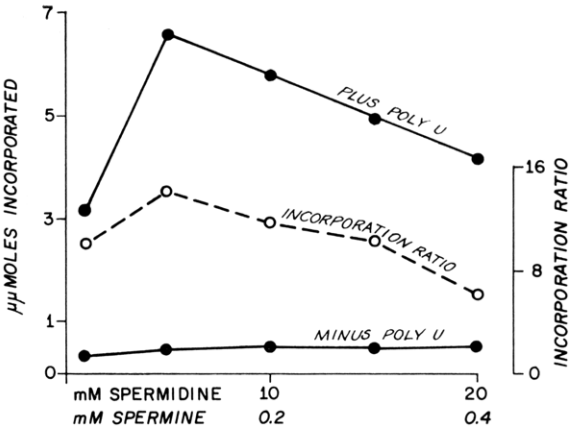

FIGURE 10: Effect of polyamine mixture on poly U directed phenylalanine incorporation. Each reaction mixture contained in a total volume of 0.1 ml: 0.1 M ammonium cacodylate (pH 7.2), 0.013 M $Mg(OAc)_2$, 0.003 M PEP, 20 μg/ml of pyruvate kinase, 0.00006 M GTP, 0.48 OD_{260} unit of poly U when indicated, 46.5 $\mu\mu$moles of yeast [^{14}C]phenylalanyl-tRNA, and a 1:2 dilution of preincubated S-30. The mixtures were incubated at 30° for 45 min before precipitation with 5% trichloracetic acid. After heating the suspended precipitate at 90° for 15 min, the precipitate was filtered and dried, and its radioactivity was measured.

The binding of phenylalanyl-tRNA with poly U showed rather different characteristics (Figure 9). Even without spermidine excellent binding was observed (binding ratio, 11.2), while at the optimal polyamine concentrations (5 mM spermidine and 0.1 mM spermine) the binding ratio reached 30, an order of magnitude higher than the binding ratio obtained with other synthetic messengers. Moreover the binding was very strong, approximately four times more phenylalanyl-tRNA being bound to ribosomes with poly U than the amount of lysyl-tRNA bound to an equivalent quantity of ribosomes by poly A. Poly U was unique among the messengers which were tested in having the above characteristics.

Effect of Polyamines on Polypeptide Synthesis. Bretthauer *et al.* (1963) noted that spermidine and spermine stimulate the poly U directed incorporation of phenylalanine in a yeast cell-free system. The effect of the 50:1 mixture of spermidine and spermine on poly U directed phenylalanine incorporation in an unresolved yeast cell-free extract is shown in Figure 10. Optimal incorporation was obtained with 5 mM spermidine and 0.1 mM spermine. Poly A stimulated lysine incorporation was found to be marginal even when polyamine was present, while no significant stimulation of proline incorporation could be obtained with poly C.

The stimulations of amino acid incorporation reported for yeast cell-free systems (see, for example, Downey *et al.*, 1965; Heredia and Halvorson, 1966) have been low compared with those observed in *E. coli* systems (Nirenberg and Matthaei, 1961; Gardner *et al.*, 1962; Wahba *et al.*, 1963). As the preceding evidence has shown that the binding capability of yeast ribosomes in the presence of polyamines is not very different from that of *E. coli* ribosomes, it would seem probable that the loss in activity of yeast protein synthetic systems occurs at the step where the polypeptide bond is formed, either because of the lability of the enzymes involved or because a factor necessary for efficient completion of this step is missing from the cell-free systems which have been described.

Mode of Action of Polyamine. Several workers have

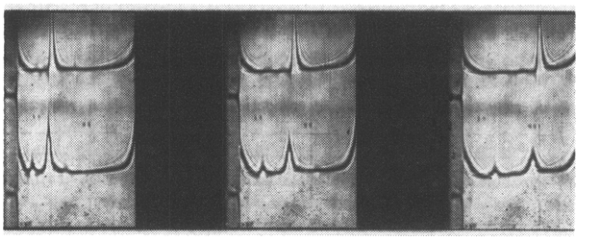

FIGURE 11: Effect of polyamines on sedimentation of yeast ribosomes. Sedimentation was carried out at 42,040 rpm in the Spinco Model E ultracentrifuge. Sedimentation was from the left to the right, photographed at 4, 8, and 12 min after reaching speed. The upper traces show the sedimentation of 4 mg/ml of ribosomes in 0.01 M $Mg(OAc)_2$, 0.05 M KCl, and 0.1 M Tris-OAc (pH 7.2). The lower photos show the sedimentation of ribosomes under identical conditions except the solution was made 20 mM spermidine and 0.4 mM in spermine.

TABLE III: Effect of Polyamine Mixture on Binding to Yeast and *E. coli* Ribosomes.

Polyamine	Mg^{2+} (mM)	Trinucleoside Diphosphate	Yeast Ribosomes[a] Yeast Tyr-tRNA[c] μμmoles Bound	Ratio[e]	*E. coli* Ribosomes[b] Yeast Tyr-tRNA[c] μμmoles Bound	Ratio[e]	*E. coli* Tyr-tRNA[d] μμmoles Bound	Ratio[e]
		None	0.46		1.42			
None	10			1.24		1.49		
		UAU[f]	0.57		2.10			
		None	1.31		2.60		0.44	
None	30			1.31		2.45		2.32
		UAU[f]	1.71		6.32		1.03	
20 mM spermidine plus		None	1.21		2.36		0.165	
0.4 mM spermine	10			4.1		2.85		2.42
		UAU[f]	4.96		6.65		0.40	

[a] Yeast ribosomes (2.05 OD_{260} units) were used in each assay. Incubation was for 20 min at 20°. [b] *E. coli* MRE 600 ribosomes (3.05 OD_{260} units) were used in each assay. Incubation was at 25° for 30 min. [c] Yeast [^{14}C]tyrosyl-tRNA (32 μμmoles) was used in each assay. [d] *E. coli* [^{14}C]tyrosyl-tRNA (11.7 μμmoles) was used in each assay. [e] Calculated binding ratio. [f] 0.305 OD_{260} unit.

reported that spermidine is capable of recombining the dissociated ribosomal 30S and 50S subunits of *E. coli* to form the complete 70S particles (Cohen and Lichenstein, 1960; Pestka, 1966).

The data in Table III show that the polyamines act on the yeast ribosome in stimulating binding. The polyamine mixture markedly stimulated the binding of yeast tyrosyl-tRNA to yeast ribosomes, while binding of the same yeast tRNA or of *E. coli* tyrosyl-tRNA to *E. coli* ribosomes was hardly affected by the polyamine mixture. The stimulating action of the polyamine on *E. coli* ribosomes at 10 mM magnesium ions is similar to the stimulation of binding to *E. coli* ribosomes which is observed when the magnesium ion concentration is raised to 30 mM. In this case the polyamine appears to act by substituting for magnesium ions. There is evidence that, *in vivo*, spermidine can substitute for magnesium ions in *E. coli* ribosomes (Hurwitz and Rosano, 1967). The effect of the polyamine on yeast ribosomes is clearly different in that magnesium ions do not substitute for the polyamine, suggesting that a specific requirement for polyamine separate from the divalent cation requirement may exist in this case. The data also preclude the possibility that the polyamine protects the aminoacyl-tRNA from amino acid hydrolysis, leading to an apparent stimulation in binding.

The fact that *E. coli* ribosomes show no specific polyamine requirement for activity may indicate some dissimilarity in structure between the yeast ribosome and the *E. coli* ribosome. These ribosomes are known to differ in their sedimentation properties and RNA content (Petermann, 1964).

The activating effect of the polyamines on the yeast ribosomes does not, however, arise by recoupling of dissociated ribosomal subunits or through a change in the state of aggregation of the ribosomes. The sedimentation patterns of the ribosomes in the ultracentrifuge when the polyamine mixture was present and absent are shown in Figure 11. In the absence of the polyamines the ribosomes sedimented as a single major component with an uncorrected s_{20} = 75 S, but when the polyamines were present two major components were observed which had uncorrected s_{20} values of 70 and 32.5 S. In both cases the large particle probably corresponds to the 80S yeast ribosomal particles observed by other workers. The decrease in the s_{20} value of the major component when the polyamine was present is probably due to an increase in viscosity in the sedimenting solution associated with the high polyamine concentration.

Discussion

Ribosomes extracted from yeast growing in the logarithmic phase were not very active in the trinucleotide-stimulated binding of aminoacyl-tRNA to ribosomes. The ribosomes as extracted were complete 75S units, and there was no evidence of ribosomal degradation into smaller subunits. The activity of the ribosomes in the binding reaction was greatly stimulated by spermidine, maximum stimulation being observed at a polyamine concentration of 20 mM. This stimulation was relatively specific and the other polyamines tested differed considerably in their effect on the binding reaction. The characteristics of the polyamine stimulated system were similar to those of the system from *E. coli* except that the magnesium ion requirement for the optimal binding ratio was lowered to 0.01 M magnesium ions. The extent of binding and binding

ratios observed in this system were also comparable to those obtained with the *E. coli* system. The action of the polyamine was demonstrated to occur at the ribosome but activation by polyamine was not accompanied by a change in the state of aggregation of the ribosome.

Polyamines occur in relatively large amounts in most organisms, including yeast (Tabor and Tabor, 1964), and extracted ribosomes have been found to contain polyamines (Cohen and Lichenstein, 1960; Spahr, 1962; Ohtaka and Uchida, 1963; Keller *et al.*, 1964; Moller *et al.*, 1965). Although the possibility that the polyamines associated with ribosomes are the result of secondary redistribution of these compounds during cell disruption cannot be entirely excluded (Cohen and Lichenstein, 1960; Siekevitz and Palade, 1962; Kim, 1966; Tabor and Kellogg, 1967), the stabilizing effects of polyamines on ribosomes and their ability to associate ribosomal subunits are well documented (Cohen and Lichenstein, 1960; Colbourn *et al.*, 1961; Martin and Ames, 1962; Ohtaka and Uchida, 1963), as are the effects of polyamines on protein synthetic systems from several organisms (Hershko *et al.*, 1961; Nathans and Lipmann, 1961; Martin and Ames, 1962; Bretthauer *et al.*, 1963; Ohtaka and Uchida, 1963; Moller and Kim, 1965). The distribution of ribosomal polyamines appears to differ in each organism, and in the cases where the effects of several polyamines on protein synthetic systems have been compared (Hershko *et al.*, 1961; Martin and Ames, 1962; Bretthauer *et al.*, 1963), the systems from various organisms differ in their response to each polyamine. The accumulated evidence suggests that polyamines may be components of the cellular protein synthetic system of many organisms.

In the brewer's yeast described here the involvement of spermidine in the cellular protein synthetic mechanism appears likely, but it is more difficult to define the role, if any, of the other polyamines, particularly putrescine and spermine, which have also been found associated with yeast ribosomes (Ohtaka and Uchida, 1963). It has recently been reported that RNA synthesis in *E. coli* is stimulated by spermidine and inhibited by putrescine (Raina and Cohen, 1966). The level of RNA synthesis appears to be controlled by the intracellular concentration of spermidine and putrescine. In the light of this finding, it is possible that a similar mechanism may control protein synthesis in yeast, as spermidine stimulates and putrescine inhibits binding at all concentrations (Figures 1 and 4). Although an extrapolation of the results of Raina and Cohen from *E. coli* to yeast may not be entirely justifiable, one could envisage mechanisms in which the intracellular concentrations of spermidine and putrescine could control both the activity of the ribosome for protein synthesis and the RNA synthetic system. The "active" and "sluggish" ribosomes found by Dietz *et al.* (1965) may, in fact, reflect the intracellular polyamine concentrations at the different stages in the growth cycle at which the ribosomes were extracted. In this connection it would be of interest to determine the effect of various mixtures of spermidine and putrescine on binding in the yeast system and to investigate the effect of the polyamines on RNA synthesis in yeast.

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Electron Microscopic Study of the Base Sequence in Nucleic Acids. VI. Preparation of Ribonucleic Acid with Marked Guanosine Monophosphate Nucleotides*

Harold Erickson and Michael Beer

ABSTRACT: It has been previously shown that the compound 2-diazo-*p*-benzenedisulfonic acid can be treated with deoxyribonucleic acid (DNA) at pH 9 to form an addition product specifically with the guanosine monophosphate (GMP) nucleotides. These can then be stained with uranyl acetate for identification of the GMP nucleotide sites by electron microscopy. Further investigaton of the chemical reaction, reported here, has shown that the reaction at pH 9 also causes an acid-labile attachment of the diazonium marker to adenosine monophosphate (AMP) and cytidine monophosphate (CMP) nucleotides. This attachment is reversed by the acid precipitation which had been used for purifying the marked DNA. A much milder acid treatment, involving incubation at pH 4, has been developed to reverse this attachment with less damage to the nucleic acid and the marked GMP. The selectivity of the reaction with ribonucleic acid (RNA) was found to be slightly less than with DNA. When 60% of the GMP's have been marked, about 10% of each of the other nucleotides is also marked. The RNA molecules are somewhat fragmented during the reaction as found by sedimentation analysis and by measuring molecular length by electron microscopy. Using sucrose gradient sedimentation to fractionate the molecules according to size it has been possible to purify small amounts of marked MS-2 RNA molecules which are more than one-half their native length. The preparation of specimens for electron microscopic analysis is described.

A method has been proposed (Beer and Moudrianakis, 1962) for determining the base sequence of nucleic acids by examining single strands at high resolution in the electron microscope. The method requires the development of reagents, called "markers," which attach selectively to certain bases in the nucleic acid, and which are, or can be made, visible in the electron microscope. For example, one such marker that has been described is the compound 2-diazo-*p*-benzenedisulfonic acid (Moudrianakis and Beer, 1965a,b). It reacts with the nucleotides, and under certain conditions primarily with GMP,[1] to form addition products. Nucleic acid which has been treated with this reagent can be extended on thin carbon film and stained with uranyl acetate, so that each of the sulfonic acid residues binds complexes of uranyl ions, thus making the markers visible in the electron microscope. High-resolution electron microscopy has demonstrated that individual markers can be identified (Moudrianakis and Beer, 1965b; P. Bartl, H. Erickson, and M. Beer, to be published).

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[1] Abbreviations used: GMP, guanosine monophosphate; AMP, adenosine monophosphate; CMP, cytidine monophosphate; UMP, uridine monophosphate; TCA, trichloroacetic acid.