Inhibition of Binding of Transfer Ribonucleic Acid to Ribosomes by Ribosomal Proteins*

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ABSTRACT: Proteins extracted from ribosomes of *Escherichia coli* inhibited the poly U directed binding of [^{14}C]Phe-tRNA to salt-washed ribosomes of *E. coli*. When the salt-washed ribosomes isolated from strains B or A-19 (endonuclease I$^-$) were first preincubated with poly U, proteins extracted from 50S subunits, but not those extracted from 30S subunits, exhibited inhibitory activity. When 50S subunits from the A-19 strain were fractionated into a "split" and "43S core" fraction by treatment with CsCl, inhibitory activity remained associated with the "43S core" proteins. The inhibitory activity of unfractionated 50S protein preparations was essentially destroyed by pepsin, but was unaffected by heating to a temperature of 80° at pH 2.0 for a period of 30 min.

The contribution of ribosomal proteins to ribosomal function is becoming increasingly clear (Nomura and Traub, 1967; Traub and Nomura, 1969). Reconstruction of functional ribosomes from subparticles and even from isolated RNA and protein indicate that the 30S subunit possesses both essential and dispensable proteins (Traub and Nomura, 1968; Traub *et al.*, 1968). Less extensive studies of reconstitution and function of the 50S ribosomal subunit have led to similar conclusions (Traub and Nomura, 1968). Studies of artificially reconstituted subunits, and kinetic analysis of the reconstitution process, indicate that essential ribosomal proteins participate in two ways: first, to perform specific binding functions, enzymatic or otherwise and, second, to provide a minimal adequate ribosome configuration (Traub *et al.*, 1968). Dispensable but stimulatory proteins are thought to determine a preferred ribosome configuration for optimal ribosome function.

The generally accepted view of ribosome assembly is that "equivalent" amounts of rRNA and of the various specific ribosomal proteins interact through a series of stepwise reactions to yield functionally active particles. However, this concept has been modified to some extent by findings which suggest heterogeneity in protein composition among ribosomes (Hardy *et al.*, 1969; Delius and Traut, 1969). The partial reversibility of the ribosome assembly process and the view that ribosome populations may be heterogeneous in protein composition suggest the possibility that ribosome function could be altered by the addition or removal of specific ribosomal proteins.

In a study of cell-free protein synthesis carried out in extracts of both exponentially growing and valine-starved cultures of *Streptococcus faecalis* ATCC 9790, we found that 105,000*g* supernatants from the amino acid-starved cells contained proteins that inhibited cell-free protein synthesis (Daneo-Moore and Shockman, 1966). In amino acid starved cells, the appearance of inhibitory activity in supernatants was accompanied by an increase in the soluble protein content and a decrease in ribosomal proteins (Daneo-Moore and Shockman, 1969). The inhibitory activity in amino acid starved *S. faecalis* supernatants was fractionated into four basic peaks by chromatography on DEAE-Sephadex. The same basic protein peaks inhibited: (a) protein synthesis directed by a natural endogenous mRNA in *S. faecalis* and *E. coli* extracts, (b) the polyuridylic acid (poly U) directed synthesis of polyphenylalanine in *E. coli* extracts, and (c) the poly U directed binding of [^{14}C]Phe-tRNA to salt-washed *E. coli* ribosomes (Daneo-Moore and Shockman, 1966, 1969). In a separate study, it was demonstrated that starvation for an essential amino acid resulted in the loss of a limited number of specific proteins from the 50S subunit of *S. faecalis* (Ziegler, 1969; R. J. Ziegler and L. Daneo-Moore, manuscript in preparation).

A simple hypothesis accounting for our findings is that the inhibitory activity found in supernatants from amino acid starved cultures of *S. faecalis* was derived from 50S ribosomal subunits. If this hypothesis were to be correct, one would expect to find the inhibitory proteins in the 50S ribosomal subunit of exponentially growing cells. In the present study we have examined the effect on the Phe-tRNA binding reaction of proteins extracted from 70S ribosomes, from 50S and 30S ribosomal subunits and from CsCl-treated subparticle cores of *E. coli*. A protein fraction which inhibits the binding of Phe-tRNA to ribosomes was isolated from a 43S core derived from a 50S ribosomal subunit of *E. coli*. The inhibitory effect of the protein fraction is exerted on ribosomes which were salt washed and preincubated with poly U.

Ribosomal protein preparations of *E. coli* rather than those of *S. faecalis* were employed for these studies for the following reasons: (1) the rather extensive knowledge of *E. coli* ribosomal proteins accumulating from work in other laboratories, (2) the recent availability of an RNase I$^-$ strain of *E. coli*

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(Gesteland, 1966), (3) unpublished results from this laboratory which indicated that an inhibitor(s) with similar properties to that of the *S. faecalis* inhibitor(s) is present in extracts of amino acid starved *E. coli* cells (Daneo-Moore and Kanefsky, unpublished data), and (4) the relative stability of *E. coli* ribosomes especially to washing in high salt concentrations.

Materials and Methods

Cell Extracts. Two strains of *E. coli* were used. Strain B (ATCC 11303) grown to early log phase and the endonuclease deficient (RNase I^-) strain A-19 grown to mid log phase. The latter is a derivative of an *E. coli* K-12 Hfr (Gesteland, 1966). The cells were purchased as a frozen paste from General Biochemicals, Chagrin Falls, Ohio.

Extracts were prepared by the method of Ron *et al.* (1966). Fifty grams (packed wet cell weight) of cells was suspended in 100 ml of cold standard buffer (0.01 M Tris-HCl, pH 7.8, 0.014 M magnesium acetate, 0.06 M potassium chloride, and 0.006 M β-mercaptoethanol) containing 50 mg of lysozyme (9900 units/mg; Worthington Biochemical Corp.). Deoxyribonuclease (Worthington Biochemical Corp.) was added at a level of 2 μg/ml. The suspension was frozen in acetone and solid carbon dioxide and then thawed in cool water until the last bit of ice was melted. After two such cycles, lysis was completed by the addition of 2 ml of 10% sodium deoxycholate, followed by incubation for 5 min at 0–4°. When necessary, the pH was readjusted to 7.8 by the addition of 0.1 N NH_4OH and the unbroken cells and debris were removed by centrifugation at 30,000*g* for 25 min.

Ribosomes. Ribosomes and their subunits were prepared by the method of Petska (1968a). The ribosomes were pelleted from the crude extracts at 105,000*g* for 4 hr. After one wash in standard buffer, the ribosomes were washed four times in 1 M NH_4Cl–0.01 M Tris-HCl (pH 7.8) and 0.01 M magnesium chloride by suspending the particles in the buffer–salt solution for 3–16 hr at 4° between sedimentations. After each resuspension aggregates were removed by centrifugation at 30,000*g* for 10 min. The washed ribosome suspensions were then centrifuged at 105,000*g* for 4 hr. Finally, the ribosomes were suspended in 1 M NH_4Cl–0.01 M Tris-HCl (pH 7.8) and 0.01 M magnesium chloride, and stored at −20° until used. The concentration of ribosomes was adjusted to about 500 A_{260} units/ml.

Ribosomes were dissociated into 50S and 30S subunits by overnight dialysis against 0.01 M Tris-HCl (pH 7.8) and 0.001 M magnesium acetate. Ribosomal 50S and 30S particles were prepared either by zone centrifugation or by differential centrifugation, as reported by Takanami (1967). For zonal centrifugation linear 10–30% sucrose gradients were prepared in 0.01 M Tris-HCl (pH 7.8) and 0.001 M magnesium acetate. One milliliter, containing up to 260 A_{260} units of dissociated ribosomes, was applied to each 50-ml gradient and centrifuged in a Spinco SW 25.2 rotor for 16 hr at 22,000 rpm at 5°. The 50S and 30S peaks were concentrated by the addition of ammonium sulfate to 80% saturation. For differential centrifugation, dissociated ribosomes were centrifuged in the Spinco S40 rotor at 39,000 rpm for 6 hr, and the upper four-fifths of the supernatants was discarded. The residual material was gently shaken for a few seconds to resuspend the upper layer of the pellet. The resuspended fraction was diluted with 0.01 M Tris-HCl–0.001 M magnesium acetate buffer (pH 7.5) and centrifuged at 39,000 rpm for 5 hr. The upper four-fifths of the supernatant was removed and the pellet was again gently shaken in the remaining supernatant (crude 30S fraction). Separately, the remaining pellet of the first centrifugation was resuspended in the same buffer and centrifuged at 39,000 rpm for 2 hr. The pellet was resuspended in a small volume of buffer (crude 50S). As determined by sucrose gradient centrifugation, 30S particles were relatively free of 50S subunits, but 50S particles were contaminated with 30S subunits to the extent of 5–10%.

Extraction of Ribosomal Proteins. Ribosomal proteins were extracted by the procedure of Fogel and Sypherd (1968). Five milliliters of cold 2-chloroethanol (Matheson, Coleman and Bell, Norwood, Ohio) was added to 1 ml of ribosomes or of ribosomal subunits (200–250 A_{260} units/ml). HCl was added to a final concentration of 0.06 N. The mixture was placed at 0° for 3 hr and vigorously shaken periodically. The precipitated RNA was removed by centrifugation at 10,000*g* for 10 min and the supernatant, containing protein, was dialyzed overnight against water to remove the 2-chloroethanol. The dialyzed solution was lyophilized and the resulting powder was used as a source of ribosomal protein. The material was contaminated with 3–4% RNA, as estimated from the $A_{280/260}$ ratio. The protein recovery averaged 70–75% of the theoretical estimate. For individual experiments the lyophilized powder was dissolved in distilled deionized water at a concentration of 4 mg/ml. The pH of the water was 5.2.

Binding. The binding of [^{14}C]Phe-tRNA to ribosomes was determined by the method of Petska (1968b). Each 100-μl reaction mixture contained 0.05 M Tris-acetate (pH 7.8), 0.05 M potassium chloride, 0.02 M magnesium acetate, 0.1 M ammonium chloride, 50 μg of poly U (Miles Laboratories, Inc.), 30 μg of *E. coli* tRNA labeled with [^{14}C]Phe (New England Nuclear; 0.005 μCi/assay, 0.191 μCi/mg), and 2.5 A_{260} units of salt-washed ribosomes. After incubation at 24° for 10 min, 0.1 μg of pancreatic RNase was added, in a total volume of 10 μl. Incubation was continued for an additional 5 min. Reactions were stopped by adding 3 ml of ice-cold 10% trichloroacetic acid to precipitate the bound [^{14}C]Phe-tRNA. The contents of each tube were then collected onto a glass fiber filter (Reeve Angel, 984H) and tube and filter were washed three times with 3-ml portions of 10% trichloroacetic acid. The filters were digested in 0.5 ml of NCS solubilizer (Amersham-Searle Corp.) for 30 min, mixed with 5 ml of scintillator fluid (toluene, 1000 ml, 1,4-bis[2-(5-phenyloxazole)]benzene, 0.1 g, and 2,5-diphenyloxazole, 4.0 g), and counted in a Nuclear-Chicago Mark I liquid scintillation spectrometer at an efficiency of about 60%.

Ribonuclease Assay. RNase was assayed by a procedure described previously (Daneo-Moore and Shockman, 1969). The assay is based on loss of acid-precipitable counts from [^{14}C]Phe-tRNA under conditions idential with those of the binding assay, except for the omission of ribosomes. Usually, RNase assays were performed in a 50 μl, rather than a 100 μl, volume. It was shown previously that with pancreatic RNase (Worthington Biochemical Corp.) this assay is linear with added enzyme between 7×10^{-3} and 1.3×10^{-2} μg of enzyme. Further, we also showed that under identical conditions this assay gives essentially the same results as

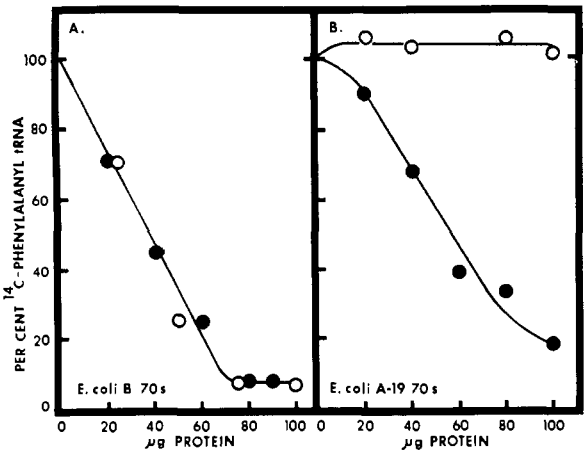

FIGURE 1: Effects of added 70S protein to the binding (●—●) and RNase (○—○) assays. (A) Proteins extracted from salt-washed *E. coli* B ribosomes. The control assay using *E. coli* B ribosomes gave 1788 cpm bound and 2000 cpm of acid-precipitabie [^{14}C]Phe-tRNA. (B) Proteins extracted from the RNase I$^-$ A-19 strain. The control assay using *E. coli* A-19 ribosomes gave 1782 cpm bound and 2221 cpm of acid-precipitable [^{14}C]Phe-tRNA.

the [^{3}H]poly U assay described by Salas *et al.* (1965) and that the two assays are linear with loss of binding activity.

Results

Inhibitory Activity of Proteins Extracted from Salt-Washed 70S Ribosomes. Proteins extracted from 70S ribosomes of either *E. coli* or A-19 inhibited the binding of Phe-tRNA to *E. coli* B and A-19 ribosomes, respectively (Figure 1). However, the *E. coli* B 70S ribosomal proteins contained a marked RNase activity which closely paralleled binding inhibition (Figure 1A). RNase activity could not be detected in the proteins from the A-19 ribosomes although the binding assay showed inhibition (Figure 1B). The inhibition of and absence of detectable RNase activity of the A-19 70S proteins suggested that inhibition of binding in this case cannot be attributed to RNase activity.

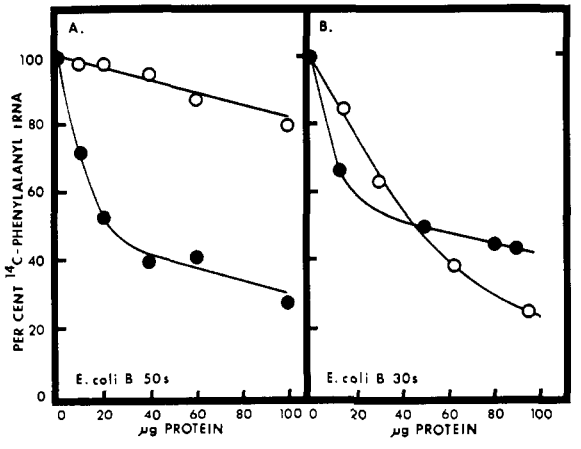

FIGURE 2: Proteins from *E. coli* B. (A) 50S subunit protein and (B) 30S subunit protein. Assay conditions as in Figure 1. Binding assay (●—●) and RNase assay (○—○). Control assays gave 1466 cpm bound and 8788 cpm of acid-precipitable [^{14}C]Phe-tRNA.

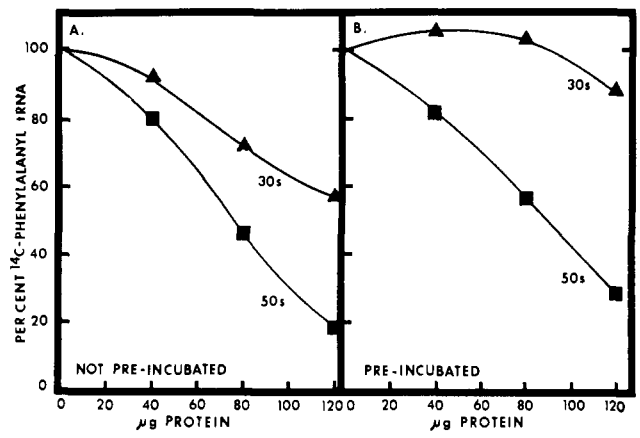

FIGURE 3: Inhibition of binding obtained from 30S (▲—▲) and 50S (■—■) A-19 proteins. In part A the order of addition was buffer-salts, protein, ribosomes, poly U, and [^{14}C]Phe-tRNA. In part B ribosomes and poly U were preincubated for 5 min in salts at 24° prior to the addition of proteins and [^{14}C]Phe-tRNA. Control assays gave 3948 and 4484 cpm bound for parts A and B, respectively.

Inhibitory and RNase Activity of Proteins Extracted from Isolated 30S and 50S Subunits. 30S proteins from *E. coli* B inhibited binding and contained RNase activity (Figure 2B), while the proteins from the 50S subunit inhibited binding but had little RNase activity (Figure 2A). The concentration of 50S protein which inhibited Phe-tRNA binding by 50% caused hydrolysis of only 5% of the Phe-tRNA in the RNase assay. This level of RNase activity could be accounted for by the 5–10% contamination of the 50S subunit preparation with 30S particles.

Proteins from both 30S and 50S subunits from the A-19 strain inhibited the binding reaction (Figure 3A). However, when the ribosomes were first preincubated with poly U (Figure 3B) the inhibitory activity of the 50S proteins was not significantly changed but the 30S proteins failed to inhibit. Since the inhibitory activity of the A-19 30S proteins could not be attributed to RNase activity (Figure 2) this result suggests that the 30S protein fraction also contains a binding inhibitor(s). This inhibitor(s) differs from that

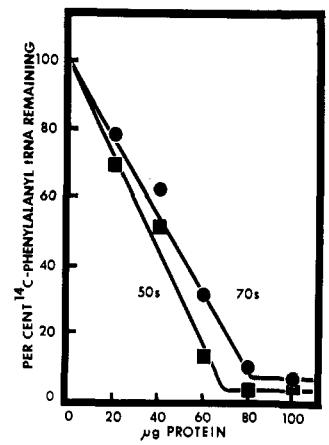

FIGURE 4: Inhibition obtained from A-19 70S or 50S proteins using freshly prepared A-19 assay ribosomes. The control reaction bound 5512 cpm or [^{14}C]Phe-tRNA.

TABLE I: Effects of 50S and 30S A-19 Proteins on the Binding of [^{14}C]Phe-tRNA to Salt-Washed Ribosomes in the Absence of Poly U (Nonspecific Binding).

Reaction Conditions	cpm
No RNase; [^{14}C]Phe-tRNA control	6397
Plus RNase; [^{14}C]Phe-tRNA control	60
Complete binding assay system	
+Poly U	6512
−Poly U	214
−Poly U + 50S protein, 100 μg	230
−Poly U + 50S protein, 200 μg	505
−Poly U + 30S protein, 100 μg	214
−Poly U + 30S protein, 200 μg	214

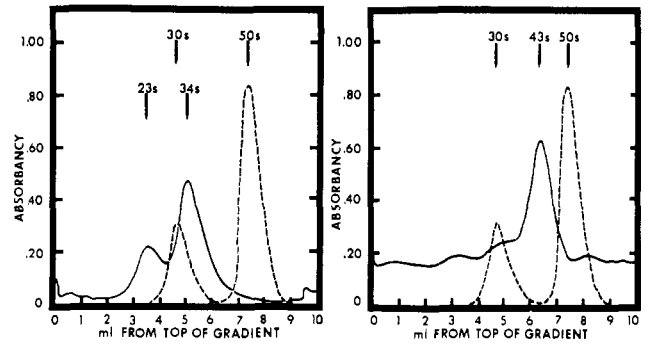

FIGURE 5: Sucrose gradient centrifugation of CsCl-treated 50S subunits of *E. coli* A-19. (A, right) Subparticles obtained after incubation of 1.0 A_{260} unit of ribosomes in 1.0 ml of 5 M CsCl–0.001 M magnesium acetate for 60 hr at 4°. (B, left) Subparticle obtained after incubation of 0.7 A_{260} unit of ribosomes in 5 ml of 5 M CsCl–0.04 M magnesium acetate for 16 hr at 4°. The nominal sedimentation coefficients were estimated by the method of Britten and Roberts (1961), using the 30S and 50S subunits as markers.

in the 50S protein fraction since it was no longer effective when the ribosomes were preincubated with poly U. All subsequent assays used poly U preincubated ribosomes.

In the absence of poly U, the 50S proteins had a stimulatory effect (Table I). The 30S proteins had no effect on this nonspecific binding reaction.

Binding inhibition was usually sigmoidal in shape with increasing protein concentration. However, when 70S and 50S proteins were assayed on the same batch of freshly prepared ribosomes a linear response was obtained (Figure 4). The estimated specific activities were 1136 and 1399 units per mg of protein for the 70S and 50S proteins, respectively. One unit was defined previously as 1% inhibition/μg of protein (Daneo-Moore and Shockman, 1969). Removal of the 30S protein produced a 20% increase instead of a theoretically expected 33% increase in the poly U preincubated system.

Effects of CsCl 50S Split and Core Proteins. Inhibition of the binding reaction by core and split protein fractions prepared from 50S subunits by the method of Lerman *et al.* (1966) was examined. A core protein fraction obtained from *E. coli* A-19 contained inhibitory activity. However, the specific activity of the core protein was only twice that of the split protein (662 and 313 units per mg of protein, respectively). The fractionation procedure resulted in two subparticles (Figure 5A) with estimated sedimentation coefficients of 23 and 34 S, in general agreement with those reported by Lerman *et al.* (1966). It was thought possible that proteins released in the derivation of one subparticle but not the other were responsible for the lack of specificity of the fractionation procedure.

In order to increase the specificity of the fractionation, a modified procedure was developed. The Mg^{2+} concentration was increased to 0.04 M and the incubation time was shortened to 16 hr (Meselson *et al.*, 1964). Cores obtained under these conditions presented a major peak with a nominal sedimentation coefficient of 43 S (Figure 5B). When assayed on the binding reaction, no inhibition was obtained from the split protein fraction (Figure 6). Only the core protein contained the inhibitory activity. The specific inhibitory activity of the 43S core proteins was consistently less than that of the 50S subunit proteins.

In order to confirm the protein nature of the inhibitor, a 50S protein factor was treated with pepsin. As shown in Figure 7, incubation of 200 μg of protein with 2 μg of pepsin for 60 min reduced inhibitory activity from 84 to 24%. Pepsin treatment of a crude 50S protein fraction was preceded by heating to 80° for 30 min at a pH of 2.0 (Herriott, 1955). Table II shows that the crude protein fraction did not lose any activity under these conditions.

Discussion

The present study demonstrates that ribosomes from exponential phase cells contain proteins which inhibit the binding reaction. Inhibition by these proteins is specific

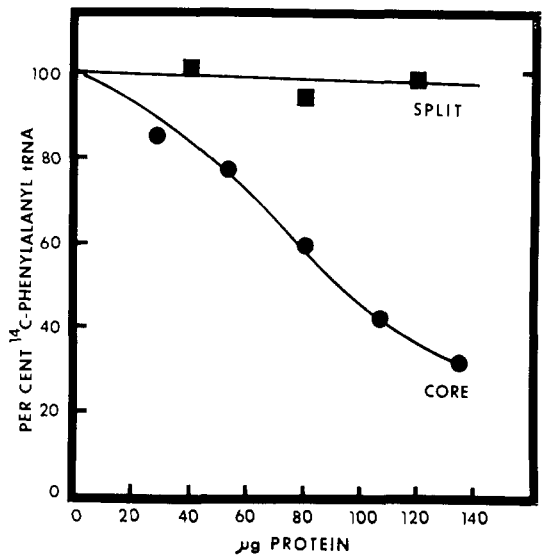

FIGURE 6: Effects on binding of split and core proteins from 50S subunits of the A-19 strain. The CsCl treatment was that used in Figure 5B. Inhibition from 43S core proteins (●—●) and from split protein (■—■). The specific activity of the core proteins was 543 units/mg of protein. The control reaction bound 5523 cpm of [^{14}C]Phe-tRNA.

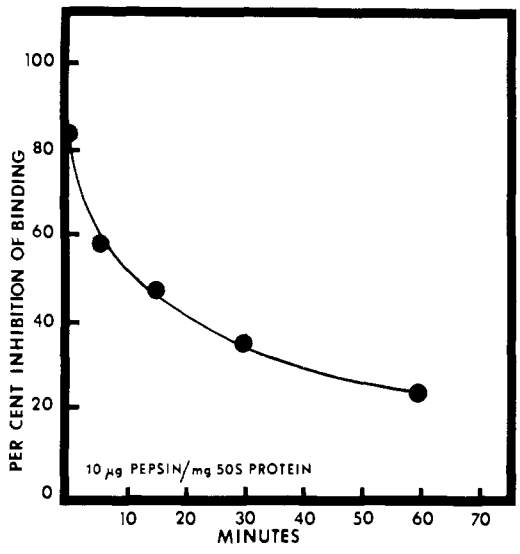

FIGURE 7: Effect of pepsin on the *E. coli* A-19 50S protein fraction. Protein (1.6 mg) was incubated at 80° for 30 min in 0.05 N HCl (pH 2.0) and treated with 16 μg of pepsin (10 μg of pepsin/mg of protein; Herriott, 1955) at 30°. At various times 200 μg of protein in a 50-ml aliquot was removed and the pH was adjusted with 5 ml of 0.5 N NaOH and 10 ml of 1 M Tris-HCl. The binding assay was run as in Figure 1.

on the basis of two criteria. First, the inhibition is effective on the poly U directed binding of Phe-tRNA to poly U preincubated ribosomes and not on the nonspecific binding of Phe-tRNA. Second, only protein from the 50S subunit and that from its derived 43S core inhibited binding to poly U preincubated ribosomes. Since 30S subunit proteins and 50S split proteins were not inhibitory, a specific class of proteins must be responsible for the inhibition.

A reduction in total inhibitory activity is obtained when ribosomes are exposed to the high salt treatment. This reduction is in the order of 50%, as estimated from the specific activity of the initial unfractionated 50S subunit protein (1399 units/mg of protein). Loss of inhibitory activity in conversion of a 50S subunit into a 43S core could be due to the splitting off of an unstable inhibitory fraction. Differential sensitivity of specific split proteins seems unlikely, however, since in studies of ribosome reconstitution a 50% decrease in function appears to occur in both the split and the core protein fractions following CsCl treatment (Nomura and Traub, 1967). Alternately, a cooperative effect from several ribosomal proteins may be required for inhibition and therefore result in decreased specific activity on fractionation of the 50S subunit.

Traub *et al.* (1968) have shown that excess split proteins derived from the 30S ribosomal subunit interfere with *in vitro* polyphenylalanine synthesis. The inhibitory effect obtained during reconstitution experiments is thought to arise from nonspecific adsorption which interferes with the stoichiometry of the reconstitution process. The inhibition of binding observed here when 30S proteins were added to untreated ribosomes may also be due to a similar phenomenon. In our study, inhibition by 30S proteins was completely blocked by preincubation of ribosomes with poly U, suggesting that the synthetic messenger conferred to the ribosome a "more

TABLE II: Effect of Bonding of 50S A-19 Proteins Heated at 80° for 30 min in 0.05 N HCl.

Reaction Conditions	cpm	% Inhibn
Control	4307	0
200 μg of protein	467	89
200 μg of protein, at 80°, 30 min, pH 2.0	501	88

stable" configuration. Inhibition by 50S proteins was not decreased by preincubation of ribosomes with poly U.

It is not clear at this time whether the inhibition by the 50S proteins is exerted on the ribosome, or whether it is due to an indirect effect on other components of the system, primarily Phe-tRNA. From studies using poly U preincubated ribosomes, it is evident that the inhibition is not exerted on formation of the 30S subunit–poly U complex.

The kinetics of inhibition from crude, or partially fractionated, ribosomal proteins can be linear or sigmoid in shape. An important factor in determining the kinetics of the inhibition is age of the assay ribosomes. Freshly prepared, highly active, salt-washed ribosomes gave a linear increase of inhibition to a maximum of over 95%. Sigmoid-shaped kinetics occurred with aged assay ribosome preparations. Increasing Mg^{2+} concentration or decreasing NH_4^+ concentration also reduced the inhibitory effect of crude proteins on the specific binding reaction (S. Sho and L. Daneo-Moore, 1969, unpublished data). These effects suggest that the target of the inhibitory effect is the ribosome.

One hypothesis explaining inhibition obtained from 50S proteins is that a poly U preincubated ribosome retains enough configurational freedom in the 50S subunit to accept additional 50S proteins which in turn affect 50S subunit function. According to this view, proteins from a 50S subunit would act directly on their homologous (50S) subunit. However, at present we have no evidence against a second answer—that these proteins can be acting on the heterologous (30S) subunit, or on the 70S monosome.

Inhibition of binding from ribosomal 50S proteins presents one unexplained feature. Even the most active preparation assayed under optimal conditions (Figure 4) required 45 μg of protein to give 50% inhibition. Since recovery of extracted ribosomal protein is 70–75% by our procedure, 110–130 μg of protein would be required theoretically for inhibition of an assay system containing only 55 μg of ribosomal protein (2.5 A_{260} units).

Partially purified basic inhibitory proteins from amino acid starved supernates of *S. faecalis* inhibited at a protein: ribosomal protein ratio of 0.049 to 0.070 (Daneo-Moore and Shockman, 1969). When this value is compared to the present ratio of 2.0 to 2.5 it is apparent that the inhibitory activity of the 50S ribosomal protein fraction must be derived from a few, possibly minor, ribosomal protein constituents. Alternately, inhibitory activity may be restricted to a small fraction of the ribosomal population. Heterogeneity in some of the minor protein constituents of the *E. coli* 50S subunit has been suggested (Delius and Traut, 1969). Attempts

were made to purify the inhibitory activity from the 50S subunit proteins of *E. coli* by methods used in this laboratory to fractionate the soluble inhibitor found in amino acid starved *S. faecalis* (Daneo-Moore and Shockman, 1969), as well as by other methods. However, we were unable to obtain a reproducible fractionation of inhibitory acitivy by (a) DEAE-Sephadex chromatography, (b) ammonium sulfate fractionation in the presence or absence of 6 M urea, (c) phosphocellulose column chromatography as described by Hardy *et al.* (1969) for 30S proteins, or (d) by gel filtration. All of these methods have been used successfully in our laboratory to fractionate inhibitory activity in soluble extracts and/or to fractionate ribosomal proteins of *S. faecalis*[1] and 30S ribosomal proteins of *E. coli*. Efforts to successfully fractionate inhibitor from 50S proteins of *E. coli* are continuing. It seems possible that, in *E. coli*, the inhibitor protein(s) interact specifically or nonspecifically with the other ribosomal proteins even in the presence of 6 M urea. Interaction of 30S ribosomal proteins has been reported recently (Mizushima and Nomura, 1970).

The present findings, together with our previous studies of changes in ribosomal protein during amino acid starvation, agree generally with the view that ribosome function can be modified by adding specific proteins. Protein-induced functional changes occur during polypeptide chain initiation and termination (see review of Lengyel and Söll, 1969). However, the inhibitory proteins found in supernatants of amino acid starved cells act on chain elongation apparently through inhibition of the specific AA-tRNA binding reaction (Daneo-Moore and Shockman, 1969). The effect of changes in the location of ribosomal proteins on the regulation of protein synthesis in nutritionally limited situations remains speculative.

[1] While fractionation of inhibitory proteins from ribosomes of *E. coli* thus far have not been successful, we have been able to fractionate a crude ribosomal fraction of exponential phase *S. faecalis* (L. Daneo-Moore and G. D. Shochman, in preparation). In the latter case, inhibitory activity was not detected in the soluble fraction but, on DEAE-Sephadex chromatography, a ribosomal protein(s) yielded peaks of inhibitory activity corresponding to peaks B, C, and D of Figure 4 in Daneo-Moore and Shockman (1969).

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