The Identification by Affinity Chromatography of the Rat Liver Ribosomal Proteins That Bind to Elongator and Initiator Transfer Ribonucleic Acids*

(Received for publication, January 18, 1980)

Norbert Ulbrich,‡ Ira G. Wool,‡ Eric Ackerman,§¶ and Paul B. Sigler¶
From the Departments of ‡Biochemistry, and of §Biophysics and Theoretical Biology, The University of Chicago, Chicago, Illinois 60637

Mixed yeast elongator-tRNAs (bulk tRNA lacking tRNA_32, pure isoaccepting species of elongator-tRNAs (tRNA_m^32 and tRNA_m^55), and purified initiator-tRNA (tRNA_h^32) were each oxidized with periodate and the 3' terminus was coupled to Sepharose 4B through an adipic acid dihydrazide spacer. The rat liver ribosomal proteins that associated with the tRNAs were isolated by affinity chromatography and identified by electrophoresis in polyacrylamide gels. The rat liver ribosomal proteins that were bound to the elongator-tRNA preparations were L6, L35a, and S15; small amounts of a number of other proteins also associated with the nucleic acid. When initiator-tRNA (tRNA_h^32) was immobilized on Sepharose, only L6 and L35a were bound; no 40 S subunit proteins associated with initiator-tRNA. No Escherichia coli proteins formed a complex with either eukaryotic initiator- or elongator-tRNAs.

The binding of aminoacyl-tRNAs to the ribosome during protein synthesis is not merely the result of codon-anticodon interaction. The aminoacyl-tRNAs associate with ribosomal proteins (1, 2) and perhaps ribosomal ribonucleic acids (3) as well. Knowledge of the identity of the ribosomal proteins that interact with tRNA is important for an understanding of the function of ribosomes, that is, of the mechanism of protein synthesis. Experiments have been carried out to determine by affinity chromatography (4-6) the rat liver ribosomal proteins that bind to tRNA. For that purpose, either mixed yeast elongator-tRNAs (cytoplasmic tRNAs lacking tRNA_32^m, or pure isoaccepting species of elongator-tRNAs (tRNA_m^32 and tRNA_m^55), or initiator-tRNA (tRNA_h^32), were immobilized on Sepharose columns and the ribosomal proteins that formed a stable association with the nucleic acids were identified by polyacrylamide gel electrophoresis.

EXPERIMENTAL PROCEDURES

The Preparation of Elongator and Initiator tRNAs—Bulk tRNA was prepared (7) from bakers' yeast (Saccharomyces cerevisiae) and the tRNA_32 was removed by fractionation on Sepharose 4B (8). Initiator tRNA (tRNA_h^32) and tRNA_m^32 were prepared from tRNA_m^32 by chromatography on DEAE-Sephadex A-50 (8). Pure tRNA_h^32 was prepared by chromatography on benzoylated DEAE-cellulose (9, 10).

Other Procedures—The following have been described before: the preparation of rat liver ribosomes (11), of ribosomal subunits (12), and of ribosomal proteins (13); the preparation of the Sepharose 4B-adipic acid dihydrazide conjugate (14, 15); the oxidation of tRNA with periodate (16) and the coupling of the oxidized tRNA to the Sepharose 4B-adipic acid dihydrazide (4); the chromatography of ribosomal proteins on the tRNA affinity columns (5, 6); the analysis of ribosomal proteins and of tRNA by polyacrylamide gel electrophoresis (5, 6); and the determination of the concentration of protein (17).

RESULTS

Affinity Chromatography of Rat Liver Ribosomal Proteins on Mixed Elongator tRNA-Sepharose—The bulk of the yeast elongator-tRNAs was resolved from tRNA_32^m by chromatography on Sepharose 4B (Fig. 1). The elongator-tRNA preparation was purified further by filtration through Sephacryl S-200 (Fig. 2) which removed small amounts (11.5%) of contaminating 5 S tRNA (Fractions 30 to 40 in Figs. 2 and 3). The purified mixed elongator-tRNAs (Fractions 50 to 62 in Figs. 2 and 3) which lacked the capacity to be aminocylated with methionine were used to construct an affinity column. Yeast, rather than rat liver, tRNA was used because of the availability of the relatively large amounts of pure isoaccepting species needed to prepare affinity columns. Yeast aminoacyl-tRNAs are used efficiently by mammalian ribosomes in the catalysis of protein synthesis (21, 22) and, hence, are likely to associate with homologous rat liver ribosomal proteins.

In separate experiments, mixtures of all of the proteins of one of the subunits of rat liver ribosomes were chromatographed (in binding buffer) on elongator-tRNA-Sepharose columns at 22°C (Fig. 4). In these experiments, the elongator-tRNA is in excess; the ribosomal proteins are in limiting amounts. The proteins that were bound to elongator-tRNA were eluted with buffer containing a high concentration of salts and EDTA (dissociation buffer), collected, and identified by a combination of one-dimensional electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate (23, 24) and electrophoresis in two dimensions in gels containing urea (25-27). The combination of the two procedures is important. There are pairs of ribosomal proteins that can not be identified by either method alone; however, a certain identification can always be made by combining the two (6).

In the first experiment, the proteins of the large subunit of rat liver ribosomes were chromatographed on elongator-tRNA-Sepharose columns (Fig. 4A). The proteins that were bound to elongator-tRNAs, about 4% of the total, were collected and a preliminary identification made after separation by electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate (Fig. 5). The stained gel was scanned at 540 nm; there were two major peaks (Fig. 5). The approximate molecular weight of the proteins in the bands was 13,700 and 33,000. The bands were aligned with a gel of all of the proteins of the 60 S ribosomal subunit; the 33,000 molecular weight
Ribosomal Proteins That Bind to tRNA

FIG. 1. Chromatography on Sepharose 4B of bulk yeast tRNA. A sample (76,200 A260 units) of bulk bakers' yeast tRNA, of which 2.2 µmol could be aminoacylated with methionine, was dissolved in 30 ml of Buffer A (10 mM sodium acetate and 10 mM MgCl₂, pH 4.5, at 22°C) and an equal volume of 3 M ammonium sulfate and applied to a column (4 x 100 cm) of Sepharose 4B that had been equilibrated with Buffer B (1.5 M ammonium sulfate, 10 mM sodium acetate, and 10 mM MgCl₂, pH 4.5). The tRNA was eluted at 4°C with a 3.5-liter reverse salt gradient of 1.5 to 0.9 M ammonium sulfate in Buffer B. The flow rate was 90 ml/h and 14-ml fractions were collected. The column was purged with Buffer A after Fraction 232. The elution of tRNA was monitored by determining the absorption at 260 nm. The tRNA species were identified by aminoacylation with [35S]methionine (18) using a preparation of mixed E. coli aminoacyl-tRNA ligase (19, 20). The fractions that could not be acylated with methionine were pooled and the tRNA precipitated with 95% ethanol; the precipitate was washed twice with ethanol and dried in vacuo.

The protein, which accounts for about 70% of the bound material, was provisionally identified as L6; it corresponded to a band that contained that one protein. The 13,700 molecular weight protein (about 15% of the material bound to elongator-tRNAs) migrated in alignment with a band that contained L22 and L35a; thus, a certain identification required two-dimensional gel electrophoresis (Fig. 6A). The latter procedure confirmed the identity of L6 and established that the second protein was L35a (not L22). Two-dimensional gel electrophoresis also revealed that small amounts of L8 and L14 had been bound to the preparation of elongator-tRNAs (Fig. 6A).

The proteins of the 40 S ribosomal subunit were next passed through the elongator-tRNA-Sepharose affinity column (Fig. 4B). About 3.5% of the protein associated with the nucleic acid. There was one prominent band on the scan of the polyacrylamide sodium dodecyl sulfate gel (Fig. 7); it accounted for some 63% of the bound material, and it corresponded to a band that contained only S15. There were a number of less prominent bands on the scan of the gel. Two-dimensional gel electrophoresis (Fig. 6B) confirmed that S15 was the major small subunit protein that was bound to elongator-tRNA; the procedure indicated the minor proteins were S9, S13, S18, and S23/S24.

No Escherichia coli ribosomal proteins associated with eukaryotic elongator-tRNA immobilized on Sepharose.

Affinity Chromatography of Rat Liver Ribosomal Proteins on tRNA<sup>20S</sup>-Sepharose and tRNA<sup>50S</sup>-Sepharose—In some preparations of bakers' yeast, the elongator tRNAs can lack all or part of the 3'-terminal trinucleotide, CCA. For that reason, it was deemed important to repeat the experiment

<sup>1</sup>E. Ackerman, and P. B. Sigler, unpublished observation.

with elongator tRNAs whose 3'-terminal trinucleotide had been rebuilt with nucleotidyltransferase (28). Moreover, it was possible that special classes of elongator-tRNAs might associate with separate, individual ribosomal proteins; that possi-
bility would go unrecognized in the experiments in which a mixture of elongator-tRNAs had been used, but should be revealed if binding of ribosomal proteins to pure isoaccepting species of tRNA was analyzed. For that purpose, separate columns of tRNA_{Met}^-Sepharose and of tRNA_{Phe}^-Sepharose were constructed. The protocol was the same as in the exper-

FIG. 4. Affinity chromatography of rat liver ribosomal proteins on tRNA-Sepharose. In A, 22 mg of 60 S ribosomal subunit protein in 45 ml of binding buffer (20 mM Tris-HCl, pH 7.4, 0.3 M KCl, 20 mM MgCl₂, and 6 mM 2-mercaptoethanol) was applied to a column (1 x 5 cm) of Sepharose 4B-adipic acid dihydrazide to which 5 mg of elongator-tRNA had been coupled. Chromatography was at 22°C; the binding buffer had a conductivity of 37 mS at that temper-

FIG. 5. Electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate of 60 S ribosomal subunit proteins separated by affinity chromatography on elongator-tRNA-Sepharose. TP60, 45 µg of all the 60 S ribosomal subunit proteins; B, 3 µg of the proteins that were bound to elongator-tRNA in Fig. 4A. The proteins that were bound to elongator-tRNA were scanned at 540 nm. A diagram giving assignments of all the 60 S ribosomal subunit proteins to individual bands is at the top.

affinity chromatography of rat liver ribosomal proteins on tRNA-Sepharose. In A, 22 mg of 60 S ribosomal subunit protein in 45 ml of binding buffer (20 mM Tris-HCl, pH 7.4, 0.3 M KCl, 20 mM MgCl₂, and 6 mM 2-mercaptoethanol) was applied to a column (1 x 5 cm) of Sepharose 4B-adipic acid dihydrazide to which 5 mg of elongator-tRNA had been coupled. Chromatography was at 22°C; the binding buffer had a conductivity of 37 mS at that temperature. The ribosomal proteins that did not bind were collected (Peak A) and those that did associate with the nucleic acid (Peak B) were eluted with a linear gradient formed of equal parts of binding buffer and dissociation buffer (20 mM Tris-HCl, pH 7.4, 2 M KCl, 5 mM EDTA, and 6 mM 2-mercaptoethanol). In B, chromatography was of 18 mg of 40 S ribosomal subunit protein in 30 ml of binding buffer on elongator-tRNA-Sepharose just as in A. In C, 20 mg of 60 S ribosomal subunit protein in 35 ml of binding buffer was applied to a column (1 x 5.5 cm) of Sepharose 4B-adipic acid dihydrazide to which 1 mg of pure yeast initiator-tRNA (tRNA_{Met}) had been coupled. The conditions for affinity chromatography were the same as in A. No ribosomal proteins were bound to Sepharose-hydrazine.
Ribosomal Proteins That Bind to tRNA

approximately 13,700 and 33,000 in a molar ratio of about 1:2, just as was observed with elongator-tRNAs. Indeed, two-dimensional electrophoresis (Fig. 6C) confirmed the proteins were L6 and L35a. However, in contrast to the results obtained in elongator-tRNA affinity chromatography, no 40S ribosomal subunit proteins were bound to initiator-tRNA. Once again, no E. coli ribosomal proteins were bound to eukaryotic initiator-tRNA.

(Fig. 4C). The fraction that was retained, about 3.7% of the total, was eluted and analyzed by one-dimensional electrophoresis in gels containing sodium dodecyl sulfate (Fig. 9). There were two bands containing proteins of molecular weight ap-

**Table 1**

<table>
<thead>
<tr>
<th>Association of ribosomal proteins with nucleic acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>5S rRNA</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>L6</td>
</tr>
<tr>
<td>L8</td>
</tr>
<tr>
<td>L19</td>
</tr>
<tr>
<td>S9</td>
</tr>
<tr>
<td>S13</td>
</tr>
</tbody>
</table>
Ribosomal Proteins That Bind to tRNA

**Fig. 8.** Electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate of ribosomal proteins separated by affinity chromatography on tRNA_Phe-Sepharose and tRNA_Met-Sepharose. The 60 S and 40 S ribosomal proteins (3 µg) that were bound to tRNA_Phe-Sepharose and tRNA_Met-Sepharose were analyzed by gel electrophoresis. The analysis of 45 µg of all the 60 S and 40 µg of all the 40 S ribosomal subunit proteins is included for comparison. The identification of the proteins in the bands was by two-dimensional gel electrophoresis.

**Fig. 9.** Electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate of 60 S ribosomal subunit proteins separated by affinity chromatography on initiator-tRNA.

**DISCUSSION**

The aim of research on eukaryotic ribosomes is to know the structure and function of the organelle: to be able to specify the location and to define the activity of each of the molecules in the particle. The proteins and nucleic acids in ribosomes form structural and functional domains. Thus, solution of the structure and an understanding of the relationship of structure to function require information on the interaction of the ribosomal proteins with ribosomal RNA and with nucleic acid ligands that associate with ribosomes during protein synthesis. An attempt is being made to identify by affinity chromatography, a procedure introduced for this purpose by Burrell and Horowitz (4), the ribosomal proteins that interact with the several relevant species of RNA (Table I; Refs. 5, 6, and 29).

The rat liver ribosomal proteins that bind to 5 S rRNA immobilized on Sepharose have been identified as L6 and L19 (5) and those that interact with 5.8 S rRNA as L6, L8, L19, S9, and S13 (6). Those results in general confirm and extend the findings of others (29), although there remain some differences that may be related to the conditions in which the experiments were conducted, especially with respect to the concentration of ions and the temperature during affinity chromatography and to the identification of the proteins by gel electrophoresis (see Refs. 5 and 6 for a more complete discussion).

The rat liver ribosomal proteins that bind to mixed elongator-tRNAs and to pure isoaccepting species, tRNA_Phe and tRNA_Met, are L6, L35a, and S15. A number of proteins, of which L8 is perhaps the most prominent, bind to elongator-tRNAs in small amounts. It is not possible to assess their significance. They may merely be contaminants, or they may have interacted secondarily with the prominent binding proteins, or they may associate with elongator-tRNAs but their affinity for the ligand may be less than that of proteins that bind in larger amounts.

Although it is not proven, we will assume that the proteins L6, L35a, and S15 form a part of the ribosome surface that...
participates in the binding of aminoacyl-tRNA during the elongation reactions of protein synthesis. That domain may also encompass 5.8 S rRNA and the proteins associated with it, L6, L8, L19, S9, and S13 (see Refs. 3 and 6 for a discussion of the evidence). That L6 associates with both nucleic acids would indicate that elongator-tRNAs bind to the ribosome in the neighborhood of 5.8 S rRNA. If 5.8 S rRNA forms a part of the ribosomal surface (the A site) to which elongator aminoacyl-tRNAs bind, and if 5.8 S rRNA and elongator-tRNAs bind to both small and large subunit proteins, it follows that 5.8 S rRNA and at least some of the proteins that associate with the nucleic acid are located on the surface of the 60 S particle near its interface with the small subunit. In support of the assumption it has been shown that 40 S ribosomal subunits will bind to 5.8 S rRNA attached to an affinity column (30). Finally, the interpretation is consistent with a great deal of circumstantial evidence that the binding of aminoacyl-tRNA and peptide bond formation occur on the surface where the two ribosomal subunits abut.

The rat liver ribosomal proteins that associate with tRNA\(^{amino}\) immobilized on affinity columns are L6 and L35a. Once again, the assumption is made that the initiator-tRNA binds to the same proteins that it interacts with during initiation of protein synthesis, although that has not been substantiated experimentally. If the assumption is correct, then L6 and L35a should be part of the ribosomal surface designated as the P site. There are indications that the P site includes 5 S rRNA and the proteins associated with it, L6 and L19 (see Refs. 3, 5, and 6 for a discussion of the evidence). It is not entirely surprising that two proteins L6 and L35a can interact with elongator-tRNAs and initiator-tRNA. First, the two proteins could each contribute two separate binding sites: one each to the A site to bind elongator tRNAs, and one each to the P site to bind initiator-tRNA. This is consistent with the requirement that the A and P sites be close enough (within 20 Å) to allow for peptide bond formation. Perhaps a more plausible explanation derives from the observation that aminoacyl-tRNA binds successively, in a codon-specific reaction, to the A and P sites during each of the reiterative cycles of peptide bond formation (31, 32); hence, elongator-tRNAs may not bind exclusively to A site proteins in affinity experiments of the type we have carried out.

It is remarkable that L6 binds to initiator- and elongator-tRNAs as well as 5 S and 5.8 S rRNAs, and that L35a binds to both types of tRNAs and, in small amounts, to 5.8 S rRNA. That the two proteins bind to 5 S and 5.8 S rRNA may be a reflection of their proximity in the ribosome; L6 and L35a may have separate and distinct sites for the binding of several different nucleic acids.

It is perhaps especially important that the small ribosomal subunit protein S15 associated with elongator-tRNAs but not with initiator-tRNA. The finding suggests that the binding of Met-tRNA to the 40 S ribosomal subunit during formation of an initiation complex is specified by the eukaryotic initiation factor eIF-2 (33) and that the interaction with ribosomal proteins does not occur until the 60 S subunit is added to form an 80 S initiation complex. At that stage the Met-tRNA would bind to L6 and L35a in the P site. S15 may be exclusively in the A site.

The interaction of prokaryotic (E. coli) ribosomal proteins and tRNA has been analyzed by affinity chromatography (4, 34–37). In some of the experiments, the number of proteins that bind to tRNA immobilized on Sepharose is large (4, 35–37), but if careful attention is paid to the concentration of ions, only S5, S9, L1, and L17 bind to tRNA (34), a finding that is in general consistent with the results obtained using different methods (38, 39).

Metspalu et al. (29) have reported that the main rat liver ribosomal proteins that bind to unfraccionated homologus tRNA coupled to Sepharose are L6, L13, L19, L21, L26, L32, L35, L36, S6, S14, and S23/S24; a number of other proteins were bound in small amounts. Obviously, they (29) found that far more proteins associate with tRNA than are reported here. The two sets of experiments differ in several respects. Metspalu et al. (29) used unfraccionated tRNA; they did affinity chromatography at 4°C rather than 22°C, and the binding buffer they employed contained 0.2 M rather than 0.3 M KC1. Finally, the identification of the proteins was solely by two-dimensional gel electrophoresis. The use of unfraccionated tRNA probably was not critical except perhaps in the failure to discover that while S15 binds to elongator-tRNAs it does not associate with initiator-tRNA. However, the conditions employed during affinity chromatography are crucial. We have found that the binding of ribosomal proteins to nucleic acids is affected by the temperature at which the affinity chromatography is done. At 4°C, a greater number of proteins bind, perhaps because the conductance of the binding buffer is decreased, allowing nonspecific interactions, and perhaps because the lower temperature favors protein-protein interactions. The concentrations of ions in the identification of specific interactions of nucleic acids and proteins (34, 40). At lower than optimal concentrations, nonspecific interactions can occur and at higher than optimal concentrations, specific interactions may be prevented. The ionic strength of the buffer employed in the present experiments was 0.38, close to that which is optimal for the reconstitution of E. coli small ribosomal subunits (40) and for the binding of E. coli ribosomal proteins to tRNA-Sepharose (34). We have found that the concentration of ions used in these experiments was optimal for the binding of rat liver ribosomal proteins to ribosomal nucleic acids.

While Metspalu et al. (29) found a far greater number of proteins to bind to tRNA than we discovered, their list probably includes the proteins we identified (L6, L35a, and S15). The reconciliation of the results requires that one examine carefully the use of polyacylamide gel electrophoresis to identify the ribosomal proteins and bear in mind that there are pairs of proteins difficult to distinguish by two-dimensional electrophoresis alone, especially if the analysis is done without a background of all the proteins to assist in the identification. The designation is facilitated when one combines two-dimensional electrophoresis in urea and one-dimensional electrophoresis in gels containing sodium dodecyl sulfate (41, 42). There is agreement in the two sets of experiments that L6 binds to tRNA, but Metspalu et al. (29) found L35 rather than L35a and S14 rather than S15 to associate with the nucleic acid. L35 and L35a are difficult to separate by two-dimensional gel electrophoresis, especially if the analysis is done without a background of all the 60 S ribosomal proteins. However, they can be easily resolved on gels containing sodium dodecyl sulfate (cf. Fig. 5). The same is true of S14 and S15 (cf. Fig. 7).

Some 30 proteins are released from rat liver 80 S ribosomes by treatment with 1 M ammonium chloride and ethanol (43). Those 30 proteins will cause Phe-tRNA, N-acetyl-Phe-tRNA, Met-tRNA, and Met-tRNA to be retained on Millipore filters. When the proteins are separated by filtration through Sephadex G-100, the binding activity is found in a fraction that contains S10, S14, S15, S19, L35, and L36 (43). Since the individual proteins were not tested, there is no assurance that all associate with tRNA. Nonetheless, the group contains S15 which was identified here by affinity chromatography to bind to elongator-tRNAs and L35 which, because of the difficulty

---

2 N. Ulbrich and I. G. Wool, unpublished data.
proteins that interact with nucleic acids coupled to Sepharose to facilitate the identification of the eukaryotic ribosomal proteins that associate with ribosomal nucleic acids and with elongator- and initiator-tRNAs. It remains to be shown by other methods that the proteins that interact with nucleic acids coupled to Sepharose actually associate in the ribosome during protein synthesis.

REFERENCES