Communication

The Effect of Antibodies against *Escherichia coli* Small Ribosomal Subunit Proteins on Protein Synthesis by Rat Liver Ribosomes*

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Monovalent Fab fragments from immunoglobulins, directed against *Escherichia coli* small ribosomal subunit proteins, were tested for their effect on poly(U)-directed synthesis of polyphenylalanine by rat liver ribosomes. Of the 18 Fabs tested, 3, anti-S10, anti-S12, and anti-S14, inhibited polyphenylalanine synthesis; antibodies against 3 of the 21 *E. coli* ribosomal proteins (S1, S16, and S17) were not tested. The others were either without any appreciable effect or, as in the case of anti-S11 and anti-S18, inhibited far less. Antibodies against S12 and S14 (but not against S10) inhibited elongation factor 1-dependent binding of [14C]Phe-tRNA to rat liver 80 S ribosomes. The presumption is that the prokaryotic (*E. coli*) ribosomal proteins, S10, S12, and S14, possess antigenic determinants also present in eukaryotic (rat liver) ribosomal proteins; the rat liver proteins have not yet been identified.

Eukaryotic ribosomes are appreciably larger than prokaryotic particles, contain a greater number of proteins, and have an extra molecule of RNA as well (1). In addition, the proteins and nucleic acids are, on the average, larger. The difference in size and complexity is a paradox, since eukaryotic ribosomes perform the same general function, namely to catalyze the synthesis of protein; moreover, they employ appreciably the same partial reactions. If one assumes what seems intuitively most likely, namely that ribosomes arose on a single occasion, then it follows that some of the molecular components of prokaryotic and eukaryotic ribosomes are likely to be functionally, or structurally, related, or both. There are, in fact, reports of homology between *Escherichia coli* L7/L12, and eukaryotic acidic ribosomal proteins (1–7). The homology has been inferred from sequence similarities (5, 6), from immunological tests using antisera (1–4), and from inhibition of function by antibodies (1–4, 7). There was, in addition, reason to suspect that there might be other related proteins. We have now tested the possibility: the effect of monovalent Fab fragments of immunoglobulins directed against single *E. coli* small ribosomal subunit proteins was assessed for their effect on the synthesis of polyphenylalanine by rat liver ribosomes.

**EXPERIMENTAL PROCEDURES**

**Materials—** *E. coli* tRNA was obtained from Schwarz/Mann, poly(U) from Miles Laboratories, GTP and ATP from Sigma Chemicals, and [14C]phenylalanine (517 mCi/mmol) from New England Nuclear. *E. coli* tRNA was charged with [14C]phenylalanine (and no other amino acid) using an *E. coli* S100 fraction (8). Preparation of Fab fragments from immunoglobulins raised in rabbits and specific for *E. coli* 30 S ribosomal subunit proteins was described earlier (9).

Male Sprague-Dawley rats weighing about 150 g were fasted overnight and killed by decapitation, and ribosomes were prepared from the livers (10, 11); the ribosomes were incubated with puromycin (11). A crude elongation factor preparation that has both EF-1 and EF-2 activity was made from a rat liver pH 5.2 supernatant, by batch chromatography on calcium phosphate gel (12). EF-1 was prepared from the crude elongation factor preparation by filtration through Sephadex G-200 (12).

**Poly(U)-directed Polyphenylalanine Synthesis—** Rat liver ribosomes (0.2 A_{260} unit) that had been stripped of mRNA and peptidyl-tRNA by treatment with puromycin (11), and variable amounts of a Fab fragment, prepared from an immunoglobulin directed against a single *E. coli* 30 S ribosomal subunit protein, were incubated at 37°C for 10 min in 0.2 ml of TKM buffer (50 mM Tris-HCl, pH 7.6; 125 mM KCl; 7 mM MgCl₂). The mixture was cooled on ice and the following was added (in 0.3 ml of TKM buffer): GTP, 100 μm; crude elongation factor protein, 50 μg; poly(U), 100 μg; [14C]Phe-tRNA, 120 pmol (60 nCi); dithiothreitol, 300 pmol. Incubation was for 30 min at 37°C. Synthesis of polyphenylalanine increased for 30 min, at which time it was near maximal; with longer incubation there was no further appreciable increase. The reaction was stopped by adding 2 ml of 7% trichloroacetic acid. The aminoacyl-tRNA was hydrolyzed by heating for 15 min at 95°C. Polyphenylalanine was collected on glass fiber filters (A. Thomas, 934AH), washed five times with 5% trichloroacetic acid, and twice with ethanol-ether (3:2). The filters were dried and placed in glass vials containing 5 ml of scintillation fluid (13). The radioactivity was determined in a Packard Tri-Carb model 2660 spectrometer.

**EF-1-dependent Binding of [14C]Phe-tRNA to Ribosomes—** For the assay, 0.4 A_{260} unit of ribosomes was preincubated with 0.4 A_{260} unit of a Fab fragment in 0.2 ml of TKM buffer at 37°C, for 10 min. The reaction mixture was cooled, and the following (in 0.3 ml of TKM buffer) was added: EF-1, 300 μg of protein; guanyl-5'-yl methylene diphosphonate (to inhibit protein synthesis), 100 pmol; poly(U), 125 μg; [14C]Phe-tRNA, 200 pmol (100 nCi); dithiothreitol, 270 pmol. Incubation was at 37°C for 30 min. The reaction was stopped by adding 2 ml of cold TKM buffer. The ribosomes were collected on Millipore filters and washed five times with TKM buffer. The radioactivity on the filter was determined as described above.

**RESULTS AND DISCUSSION**

Monovalent Fab fragments, prepared from immunoglobulins raised in rabbits and directed against individual *E. coli* 30 S ribosomal subunit proteins, were tested for their effect on poly(U)-directed polyphenylalanine synthesis by rat liver ribosomes. The assay was selected because it is a global one. The ability to synthesize polyphenylalanine at a relatively high concentration of magnesium, in the presence of polyuridylic acid and elongation factors, provides a gauge of the capacity of ribosomes to bind aminoacyl-tRNA, to catalyze peptide bond formation, and to translocate peptidyl-tRNA; in brief, all of the reactions between formation of an initiation *The work in Chicago was supported by Grants GM-21769 and CA-19265 to I. G. W.) from the National Institutes of Health; the work in Berlin was supported by a grant from Deutsche Forschungsgemeinschaft (to G. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

*The abbreviations used are: EF-1 and EF-2, elongation factors 1 and 2.

† The author's solution and buffers was determined at 20°C.
complex and termination of peptide synthesis. The assay provides a rapid and convenient survey of the capacity of ribosomes to carry out protein synthesis.

Of the 18 Fab fragments tested, 3 (anti-S10, anti-S12, anti-S14) inhibited polyphenylalanine synthesis; the 15 others were either without any appreciable effect or had a much smaller effect (anti-S11 and anti-S18) (Fig. 1). Two anti-S14 Fab preparations, made from immunoglobulins raised in separate rabbits, were tested: only one of the two was strongly inhibitory. The first (R 160) inhibited 100% when the antibody to ribosomes ratio (A_{280} Fab:A_{260} ribosomes) was 0.5. The second (R 306) inhibited 50% but only at a ratio of 2:1 (Fig. 1). The difference in the effects of the two separate preparations on the synthesis of polyphenylalanine by E. coli ribosomes was similar: R 160 was strongly inhibitory, whereas R 306 had only a moderate effect (results not shown). In addition, it had previously been found that only three of nine antisera against E. coli ribosomal proteins L7/L12 cross-reacted with rat liver ribosomal proteins (3). The presumption is that the individual antisera contain antibodies against different subsets of determinants.

The inhibitory activity of the anti-S10, anti-S12, and anti-S14 Fab fragments varied somewhat: anti-S14 (R 160) was completely inhibitory at a Fab:ribosome ratio of 0.5; anti-S10 was also completely inhibitory, but at a ratio of 1.0; and anti-S12 was 90% inhibitory at a ratio of 1.0 (Fig. 1). In the homologous reaction, antibodies against E. coli proteins and E. coli ribosomes, inhibition of polyphenylalanine synthesis was nearly complete at a ratio of 0.3 A_{260} Fab to 0.3 A_{260} ribosomes (9), i.e. at a ratio of 1. Thus, the Fabs were as effective in the inhibition of protein synthesis by rat liver ribosomes as by E. coli particles. The molar excess of antibodies over ribosomes necessary for complete inhibition in the present experiments was approximately 10. Anti-S11 was about 45% inhibitory, and anti-S18 about 30% inhibitory, at a ratio of 1.0 (Fig. 1). The other Fab fragments tested (anti-S2, -S3, -S4, -S5, -S6, -S7, -S8, -S9, -S13, -S15, -S19, -S20, -S21) had no effect. However, it is important to bear in mind that in experiments of this type only positive results carry weight; thus, the negative finding with the other Fab fragments must be considered tentative.

Fabs directed against most E. coli small subunit proteins strongly inhibit polyphenylalanine synthesis by E. coli ribosomes; the exceptions are anti-S1, -S6, -S7, -S15, and -S16 which have a significantly smaller effect (9). Of the Fab preparations of concern here, anti-S10 and anti-S14 are strong

![Figure 1](image_url)

**FIG. 1.** Effect of monovalent Fab fragments prepared from immunoglobulins against E. coli small ribosomal subunit proteins on poly(U)-directed polyphenylalanine synthesis by rat liver ribosomes. Some of the experiments were carried out with 0.2 A_{260} unit of ribosomes in the 0.5-ml reaction mixture described under "Experimental Procedures"; others were done with 0.1 A_{260} unit of ribosomes in 0.25 ml of reaction mixture containing one-half the amount of the substrates; in either case, the amount of the Fab fragment was varied and is expressed as a ratio to the amount of ribosomes. In control experiments with no Fab (100%), the incorporation of [^{14}C]phenylalanine into polyphenylalanine was 12,900 cpm (11.2 pmol) if 0.2 A_{260} unit of ribosomes was used, and 3,824 to 5,839 cpm (3.3 to 5.1 pmol) if 0.1 A_{260} of ribosomes was used. Separate experiments with the same Fab are indicated by different symbols. The same Fab preparations have been tested with E. coli ribosomes and the results with most have been reported (14).
The reaction mixture contained 0.4 A260 unit of ribosomes and 0.4 A260 unit of Fab fragment. In control experiments with no Fab, the amount of [3H]phenylalanyl-tRNA bound to ribosomes was 900 cpm (0.82 pmol).

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<th>Fab specific for</th>
<th>[3H]Phe-tRNA bound (% control)</th>
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<tr>
<td>S2</td>
<td>107</td>
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<td>S3</td>
<td>102</td>
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<td>S4</td>
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inhibitors of initiation factor-catalyzed AUG-dependent fMet-tRNA binding. i.e. are directed against P site decoding; anti-S12 is only moderately inhibitory of this reaction. Anti-S10 and anti-S14 inhibit EF-Tu-dependent binding of Phe-tRNA to the A site as well (9).

The effect of the set of Fab fragments against E. coli small ribosomal subunit proteins, on one of the partial reactions required for polyphenylalanine synthesis—the EF-1-catalyzed binding of Phe-tRNA to 80 S rat ribosomes in the presence of poly(U)—was assessed (Table I). Anti-S12 and anti-S14 were moderately inhibitory; at an antibody to ribosomal proteins, on one of the partial reactions is moderately inhibitory of this reaction. Anti-S10 Fab had no effect; the presumption must be that the rat liver ribosomes were not tested. We are not certain why the anti-S12 and anti-S14 Fab had no effect; the presumption must be that the rat liver protein that has determinants similar to those in E. coli S10 is involved in a partial reaction of protein synthesis that follows binding of aminoacyl-tRNA. It is even possible that the E. coli ribosomal proteins share determinants with large subunit proteins in rat liver ribosomes. In other experiments, no effect of any of the Fab fragments on nonenzymatic binding of [14C]Phe-tRNA (tested at 20 mM MgCl2) or of [3H]poly(U), to rat liver ribosomes was observed (results not shown).

The presumption must be that the E. coli small ribosomal subunit proteins S10, S12, and S14 have determinants that are present in rat liver ribosomal proteins. The putative rat liver proteins do not yet have been identified. The experiments do not establish that the proteins are homologous, since in the strictest sense two proteins are homologous only if they share a common ancestral gene. It had been shown before that E. coli ribosomal proteins L7/L12 partially cross-react with eukaryotic acidic large ribosomal subunit proteins (1-4), and that antibodies against E. coli L7/L12 inhibit the function of eukaryotic ribosomes (4), just as antibodies against the related eukaryotic ribosomal proteins inhibited the function of E. coli ribosomes (2). There were indications before that prokaryotic and eukaryotic small ribosomal subunit proteins might be related. An immunoglobulin preparation obtained from an antiserum directed against a mixture of rat liver 40 S ribosomal subunit proteins inhibited polyphenylalanine synthesis by E. coli ribosomes. Moreover, an antiserum directed against rat liver ribosomal protein S6 inhibited polyphenylalanine synthesis by E. coli ribosomes. The results, while not conclusive, suggest that some small ribosomal subunit proteins may have been conserved during evolution from prokaryotes to eukaryotes.

The E. coli ribosomal proteins S10, S12, and S14 are located, at least in part, in a domain in the head of the 30 S subunit (15) that constitutes a part of the aminoacyl-tRNA binding site (15, 17), although from the antibody attachment sites, anti-S12 is most likely to interfere with codon-anticodon interaction. There is evidence that S14 (15, 19-19) and S10 (9, 19) participate in aminoacyl-tRNA binding. E. coli S12, the streptomycin protein (20), is perhaps more directly involved in initiation than elongation (21); however, the protein affects fidelity of translation (22) and hence is also likely to be involved in binding of aminoacyl-tRNA. It is noteworthy that anti-S12 and anti-S14 inhibit EF-1-catalyzed binding of Phe-tRNA to rat liver ribosomes. Thus, it is possible that the rat liver ribosomal proteins that are related to E. coli S10, S12, and S14 take part in binding of aminoacyl-tRNA.

REFERENCES

"G. Stöffler, N. Fischer, K. H. Rak, and I. G. Wool, unpublished observation."