Interactions between 30S ribosomal proteins and 50S subunits of *Escherichia coli*.

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ABSTRACT

The interaction between ribosomal proteins of the 30S subunit with intact 50S subunits was investigated. Experiments with mixtures of total 30S proteins indicated that several 30S proteins including protein S4 would form a stable complex with 50S subunits. Further work with pure S4 indicates that this protein binds stoichiometrically to the 50S subunits, probably through protein-nucleic acid interaction. The possible significance of this interaction is discussed.

INTRODUCTION

A large body of evidence is currently available which indicates that a single ribosomal protein does not function independently of the other macromolecular components of the ribosome (1,2,3). This observation has resulted in increased emphasis on determination of the spatial relationships between individual proteins and nucleic acids of the particle. The experimental elucidation of these topological and functional relationships has in general relied on interactions between chemical probes, protein cross linking, and antibody interactions with ribosomal components (4,5,6,7). No single approach has yielded optimal information and each has its limitations which usually are a consequence of the conformational fluidity of the particles and the rapid subunit dissociation and inactivation during treatment of the 70S particle.

The requirement for subunit association for protein synthesis implies that coordinate activity between the two ribosomal particles (30S and 50S) is an essential feature of polypeptide chain elongation. Therefore, an identification of the interacting 30S and 50S components localized at the inter-

face between the two particles will be essential to the development of our concept of ribosome structure and function. Below we describe experiments which have taken a different approach to the problem with respect to those proteins of the 30S subunit which interact directly with the 50S subunit under conditions used to obtain in vitro protein synthesis. We have demonstrated that under these conditions protein S4 will independently form a stable and stoichiometric complex with the 50S subunit and probably through a protein nucleic acid interaction. Protein S11 and S12 also bind to the 50S subunit, but not to 23S rRNA either in the presence or absence of protein S4.

MATERIALS AND METHODS

Isotopically labeled ribosomal components and ribosomal subunits were prepared from exponentially growing E. coli B236 cell cultures as described by Voynow et al. and Schaup et al. (8,9). The nomenclature for proteins is as described by Wittmann et al. (10).

Association experiments between ribosomal proteins and 50S subunits were performed at 37°C in A buffer (.01 M Tris HCl, .005 M MgAc, .01 M mercaptoethanol, .2 M NH_4Cl_2 , pH 7.6). This is a slight modification of the buffer reported by Wilhelm and Haselkorn which was used to assay for in vitro protein synthesis (11). Prior to use, proteins were dialyzed into PMK I (.005 M $\text{K}_x\text{H}_x\text{PO}_4$, .02 M MgCl_2 , 1 M KCl, .06 M mercaptoethanol, pH 7.6.) and 50S and 30S subunits were stored in TSM (.01 M Tris HCl, .01 M MgCl_2 , .003 succinic acid, pH 8) at -70°C generally at 200 to 300 A_{260}/ml . Scintillation counting was performed in a Packard 3300 scintillation spectrophotometer using a TX100 counting cocktail as previously described (12). Protein concentrations were determined as described by Lowry et al. and ribosome concentrations were determined spectrophotometrically (12,13).

RESULTS

In initial experiments a total extract of ^3H - 30S proteins was incubated with intact 50S subunits and layered on sucrose gradients. The results shown in Figure 1 show that

Figure 1.

Distribution ^{32}P -50S subunits (2 cpm/ugm) and ^3H -30S protein (1100 cpm/ugm) after zone centrifugation for 7 hours at 41,000 rpm through a sucrose gradient (15-30% in A buffer) in an SW 40.1 rotor. The 30S proteins and 50S subunits were incubated at 37°C for 15 minutes in A buffer prior to centrifugation.

approximately 15% of the protein added could be found associated with 50S subunits. In these experiments a sufficient amount of protein was added, using an average molecular weight of 22,000 daltons/protein and assuming that all proteins were present in unit mole quantities, to have an approximate 0.5:1 individual protein to 50S subunit stoichiometry. The amount of protein associated with the 50S subunits was sufficient to account for unit mole presence for as many as three 30S proteins. The proteins were extracted from the 50S peak isolated on sucrose gradients mixed with 200 ugm of unlabeled total 30S proteins. This extract was then subjected to two dimensional

electrophoresis as described by Kaltschmit and Wittmann (14). The gels were stained and the proteins identified and cut out and prepared for liquid scintillation counting as previously described (12). Approximately 68% of the counts were recovered in regions identified as protein S4, S11, and S12. The total count recovery was 82%. These results suggest that the presence of any one protein will not exclude the binding of either of the other two.

Purified tritiated S4 was tested for its affinity for 50S subunits. Mixtures were prepared as before with protein S4 alone and 50S subunits. These were layered on sucrose gradients for separation of bound from unbound material, the results are shown in Figure 2A, demonstrating the binding of tritiated S4

Figure 2 A - B.

A. Distribution of ^3H -S4 (873 cpm/ugm) and ^{32}P -50S subunits (5 cpm/ugm) after zone centrifugation for 17 hours at 27,000 rpm through a sucrose gradient (15-30% in A buffer) in an SW 27 rotor. The S4 and 50S subunits were incubated for 15 minutes at 37°C in A buffer prior to separation and the ratio of S4/50S in the mixture was 3.5:1.

B. A typical saturation curve for S4 bound to 50S subunits in A buffer. Samples were prepared as before with a fixed amount of 50S subunits and increasing amounts of S4.

to 50S subunits. Figure 2B shows the results of an experiment in which increasing amounts of protein S4 were added to a fixed quantity of 50S subunits with a protein to 50S subunit ratio between 0.5 to 7. The molar ratio of S4 associated with 50S subunits was calculated using the specific activity of the 50S subunits and 30S protein respectively. The results show that protein S4 binds one to one with 50S subunits even in the presence of large excesses of protein S4. To determine the level of S11 and S12 binding to 50S subunits experiments comparable to those done for protein S4 were performed; the results are shown in Figure 3. No saturation of 50S subunits was observed for either protein S11 or S12.

Figure 3.

Typical saturation curves for proteins S11 and S12 in A buffer. Samples were prepared as described in the text with a fixed amount of 50S subunits and increasing amounts of ^3H -S11 or ^3H -S12.

To test for competitive binding of the individual proteins S4, S11 and S12 mixtures with a fixed amount of ^{32}P -50S subunit and a single unlabeled competitor protein in a 4 fold molar excess of protein to subunit were prepared with increasing amounts of either ^3H -S4, ^3H -S11 or ^3H -S12. The mixtures were incubated as described earlier and unbound material was separated from bond by ultracentrifugation. Figure 4 shows the results for S12 competition with ^3H -S4 indicating no competition. The same result was observed in all such competition experiments, e.g. S11, S4, and S12 do not compete for common binding sites on 50S subunits and cooperative binding is not observed.

In a previous publication we reported that of the individual 30S proteins tested, protein S4 would form a stable complex with 23SrRNA under the appropriate conditions (12). Figure 5 shows the binding isotherm for ^3H -S4 to ^{32}P -23S rRNA. In this experiment increasing amounts of ^3H -S4 were added to a fixed quantity of ^{32}P -23S rRNA. The molar ratios of S4 to 23S were from 0.75 to 5.5. The figure shows that a plateau is reached at a protein to RNA ratio of 2:1.

Figure 4.

A typical saturation curve for ^3H -S4 bound to a fixed quantity of 50S subunits in the presence of 4 fold molar excess of unlabeled S12 (o—o). The broken line indicates the expected result if S4 and S12 were to compete equally for a common binding site.

Figure 5.

A typical saturation curve for ^3H -S4 bound to ^{32}P -23S rRNA in A buffer. Samples were prepared in A buffer with a fixed amount of 23SrRNA and increasing amounts of S4. Bound S4 was separated from unbound material by zone centrifugation in A buffer sucrose gradients.

Competition experiments were done as before except 23SrRNA was used instead of 50S subunits. These experiments indicated that the binding of S4 is unaffected by the presence of 4 to 6 molar excesses of proteins S11 and S12 separately or together. Examination of ^3H -S11 and ^3H -S12 binding to 23SrRNA under conditions used for S4 binding indicated that these proteins will not by themselves or in the presence of unlabeled S4 bind to the 23SrRNA.

DISCUSSION

In an earlier publication we had indicated that protein S4 as well as S17 would bind to 23S rRNA (12). However, the binding of protein S17 to rRNA was considered to be nonspecific and likely and to be an artifact. These experiments were done with oxidized S17, subsequent work with reduced protein indicated that it would not bind to either rRNA (15). It should be

pointed out that in our early work and that of others, the conditions used to assay for binding of individual proteins were those reported by Traub et al. (1969) for functional reconstitution of 30S subunit (16,17). The reconstitution conditions are very different from those described in this report.

The binding of S11 and S12 to the 50S subunit has not been fully explored. However, another group has reported that S11, and S12, are capable of forming a complex with 23S rRNA (18). Significant binding of S11 was not observed unless large molar excesses of the protein were present and the reported binding of S12 to 23rRNA was judged to be non-specific in ribosome reconstitution buffers (18). Using our comparatively low ionic strength conditions and low magnesium we could not demonstrate binding of either of these proteins separately or together with protein S4 to 23S rRNA. The presence of these proteins, however, has no effect on the above reported binding of S4 to 23S rRNA or 50S subunits. This result suggests that S4 binds to a unique site on 23S rRNA as well as the 50S subunit. The binding of protein S11 to 50S subunits appears to be non-specific while the S12 binding is comparatively weak. It is possible that under different conditions their association with the 50S subunits would be altered.

There seems to be a functional relationship between S4, S11, and S12 in as much as they all affect fidelity of translation in some manner. Protein S4 has been identified as the ribosomal ambiguity protein (RAM) and protein S12 is involved in streptomycin sensitivity (19,20). Burge and Kurland (1971) have reported that in one case reversion from streptomycin dependence occurs with alterations in protein S4 (21). Reconstitution of 30S subunits in the absence of S11 yields ribosomes which lack fidelity in in vitro protein synthetic systems (1). If these proteins are involved in coupling 30S and 50S subunits then the manifestation of these effects may be a consequence of functional uncoupling of the 30S and 50S subunits.

The observation that S4 will bind to 50S subunits with a stoichiometry of 1:1 and to 23S rRNA 2:1 under identical ionic conditions may imply a possibly functional role for S4 as a

conformation effector during the process of protein synthesis. Since we do not observe dimerization of 23S rRNA in the presence of S4 this suggests that there is a single recognition site on the protein for a unique sequence in 23S rRNA. The 2:1 binding of S4 to 23S rRNA indicates that there are likely to be two such sequences within the 23S rRNA separated by an undefined number of nucleotides. Depending on the functional state of the ribosome, (initiation, peptide chain elongation, etc.) one or the other of these sites may be exposed. In response, for example, to G factor or GTP hydrolysis one site could be withdrawn and the other advanced resulting in a physical reorientation of S4 and its associated components when the new binding is initiated. Such a device would provide a molecular explanation for conformational alterations within the particle during various active phases of synthesis. This manner of conformational alteration would also synchronize the two subunits with one another during protein synthesis in essence coupling their activities.

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REFERENCES

1. Nomura, M., Mizushima, S., Ozaki, M., Traub, P., and Lowry, C. (1969) in Cold Spring Harbor Symp. Quant. Biol., Vol. 34, pp. 49-61.
2. Randall-Hazelbauer, L.L. and Kurland C.G. (1973) Mol. Gen. Genet. 115, 234-242.
3. Noller, J.F., Chang, C., Thomas, G., and Aldridge, J. (1971) J. Mol. Biol. 61, 669-679.
4. Oen, H., Pellegrini, H., Eilate, El, and Cantor, C.R. (1973) Proc. Nat. Acad. Sci. 70, 2799-2803.

5. Lutter, L.C. and Kurland, C.G. (1973) *Nature New Biol.* 243, 15-17.
6. Sommer, A. and Traut, R.R. (1974) *Proc. Nat. Acad. Sci.* 71, 3946-3950.
7. Lake, J.A., Pendergast, M., Kahan, L., and Nomura, M. (1974) *Proc. Nat. Acad. Sci.* 71, 4688-4692.
8. Voynow, P. and Kurland C.G. (1971) *Biochemistry* 10, 517-524.
9. Schaup, H.W., Sogin, M., Woese, C., and Kurland, C.G. (1972) *Mol. Gen. Genet.* 114, 1-8.
10. Wittmann, H.G., Stöffer, G., Hindennach, I., Kurland, C.G., Randall-Hazelbauer, L., Birge, E.A., Nomura, M., Kaltechmidt, E., Mizushima, S., Traut, R.R., and Bickle, T.A. (1971) *Mol. Gen. Genet.* 111, 327-333.
11. Wilhelm, J. and Haselkorn R. (1971) in *Methods in Enzymology*. Vol. XX, pp. 531-536, Academic Press, New York.
12. Schaup, H.W., Green, M., and Kurland, C.G. (1970) *Mol. Gen. Genet.* 109, 193-205.
13. Lowry, O.H., Rosebraugh, N.J. Farr, A.L., and Randall, R.J. (1951) *J. Biol. Chem.* 193, 265-275.
14. Kaltschmidt, E. and Wittmann, H.G. (1970) *Anal. Biochem.* 36, 401-412.
15. Schaup, H.W., Green, M.G., and Kurland, C.G. (1971) *Mol. Gen. Genet.* 112, 1-8.
16. Traub, P. and Nomura, M. (1968) *Proc. Nat. Acad. Sci.* 59, 777-784.
17. Stöffler, G., Daya, L., Rak, K.H., and Garrett (1971a) *J. Mol. Biol.* 62, 411-414.
18. Morrison, C.A., Garrett, R.A., Zachhardt, and Stoffer, G. (1973) *Mol. Gen. Genet.* 127, 359-368.
19. Zimmermann, R.A., Garvin, R.T., and Gorini, L. (1971) *Proc. Nat. Acad. Sci.* 68, 2263-2267.
20. Ozaki, M., Mizushima, and Nomura, M. (1969) *Nature* 222, 333-339.
21. Birge, E.A., and Kurland, C.G. (1970) *Mol. Gen. Genet.* 109, 356-369.