FURTHER EVIDENCE FOR THE PARTICIPATION OF PROTEINS
S 3, S 14 AND S 19 IN tRNA BINDING TO E. COLI 30 S SUBUNITS

MARILYN SHIMIZU and GARY R. CRAVEN
The Laboratory of Molecular Biology and the Department of Genetics
The University of Wisconsin, 1525 Linden Drive, Madison, Wis. 53706, U.S.A.

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Abstract. Previous studies have shown that iodination of 30 S subunits causes inactivation for both enzymatic fMet-tRNA and non-enzymatic phe-tRNA binding activities. This inactivation was shown to be due to the modification of three to five ribosomal proteins [1]. In this report the role of these proteins in tRNA binding activity has been further studied. Purified ribosomal proteins, isolated from modified subunits, are re-assembled into otherwise unmodified 30 S ribosomes and assayed for tRNA binding capacity. The presence of modified S 3, S 14 and S 19 (S 15) in the reconstituted particle results in substantial reduction of both fMet-tRNA and phe-tRNA binding activities. This reduction in tRNA binding activity does not appear to be due to an assembly defect.

I. INTRODUCTION

We have previously reported that iodination of 30 S subunits results in the loss of tRNA binding activity [1]. The ribosomal components responsible for this inactivation were identified by the use of the ribosome reconstitution procedure of Held et al. [2]. Disassembled modified subunits were reassembled in the presence of groups of unmodified proteins to determine if a return in functional activity could be observed. In this way, proteins S 3, S 14 and S 19 were identified at the site of iodine inactivation for enzymatic fMet-tRNA binding and proteins S 1, S 2, S 3, S 14 and S 19 at the site of iodine inactivation for nonenzymatic phe-tRNA binding. Thomas et al. [3] have utilized this same basic approach to identify protein S 3 at the site of Rose Bengal inactivation for poly U binding and proteins S 2 and S 3 at the site of Rose Bengal inactivation for phe-tRNA binding.

We now extend our iodination study of the functional sites of the 30 S subunit by following a second, complementary approach in the use of chemical modification. Modified components are substituted for their unmodified counterparts in untreated subunits to determine if a loss of functional activity can be observed. This approach has the advantage that only a few selected proteins are present in a modified state, allowing for a more definitive identification of the roles of the various ribosomal proteins. Kahan et al. [4] have also utilized this approach to investigate the role of sulfhydryl-containing proteins in ribosome structure and function.
In this report, unmodified 30 S ribosomal components are reconstituted in the presence of limited groups of purified proteins extracted from iodine-inactivated 30 S particles. Ribosomes containing iodinated proteins S 3, S 14 and S 19 (S 15) function poorly in both the fMet-tRNA and the phe-tRNA binding assays, further suggesting that these proteins are at the site of inactivation. This reduction in tRNA binding activity does not appear to be due to any significant defect in ribosome assembly.

II. MATERIALS AND METHODS

NH 4Cl washed ribosomes were prepared by a modification of the procedure of Dubnoff and Maitra [5] as described previously [1]. For iodination, the 30 S subunits were incubated at 28 °C for 5 min with a stock iodine solution of 0.5 M KI, 0.05 M I2 in fMet-tRNA binding buffer (0.05 M Tris-HCl pH 7.8, 0.1 M NH4Cl, 0.01 M Mg(OAc)2) to give a 100-fold molar excess of reactive I (KI + I2) to ribosome as described previously [1].

Ribosomal RNA and ribosomal proteins were isolated from 30 S subunits by the acetic acid-urea extraction technique of Hochkeppel, Spicer and Craven [6]. Individual proteins were fractionated by phosphocellulose column chromatography as described by Hardy et al. [7]. Proteins S 15 and S 19 were available only as a mixture during the course of these experiments. We previously reported that the addition of unmodified protein S 19 results in the restoration of fMet-tRNA binding capacity, while unmodified S 15 shows no effect [1]. Therefore, although modified S 15 and S 19 were present together in the studies reported here, we assume that modified S 19 is responsible for the effects observed.

Enzymatic fMet-tRNA binding to 30 S subunits in the presence of poly (AUG) and non-enzymatic phe-tRNA binding to 30 S subunits in the presence of poly U were assayed according to the procedures described previously [1]. Reconstituted particles were assayed directly after reconstitution without prior isolation of the particle. Reconstitutions were performed according to the procedure of Held, Mizushima and Nomura [2]. A molar ratio of 1 : 1.8 of RNA to protein was routinely used.

Reconstituted 30 S ribosomes were isolated from the reconstitution mix for the poly U binding assay by precipitation at 4 °C with 0.65 volumes of absolute ethanol. Particles were resuspended in 0.2-0.4 ml of fMet-tRNA binding buffer. Undissolved material was removed by a second low-speed spin and the supernatant was incubated at 42 °C for 20 min. (3H)-poly U (purchased from Schwarz/Mann) binding activity was assayed by the millipore filter procedure of Smolarsky and Tal [8]. The dried filters were counted in 10 ml of scintillation fluid (5 g PPO, 0.1 g dimethyl POPOP/liter toluene).

Protein samples were prepared for polyacrylamide gel electrophoresis by collecting the ribosomal peak from a sucrose gradient and precipitating the particles with two volumes of cold 100% ethanol (20°С) overnight. The precipitate was pelleted at low speed and dissolved in 8 M urea. One-dimensional polyacrylamide gel electrophoresis of the proteins was performed at pH 4.5 as described by Hardy et al. [7] with the methylene bisacrylamide modifications of Voynow and Kurland [10].

III. RESULTS AND DISCUSSION

Previous studies in our laboratory demonstrated that E. coli 30 S ribosomes are inactivated for