Purification and Characterization of 50S Ribosomal Proteins of Escherichia coli

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Received June 7, 1971

Summary. Twenty-seven proteins of the 50S ribosomal subunit from E. coli have been purified by a combination of differential solubility in ammonium sulfate, ion-exchange chromatography, and molecular-sieve chromatography. The amino acid compositions, tryptic peptides and molecular weights of these proteins have been analyzed. Each protein is unique with respect to amino acid sequence and, according to chemical criteria, reasonably pure. The sum of the molecular weights of the twenty-seven proteins is 495000. This means that the 50S subunit could accommodate one copy of each protein.

1. Introduction

The isolation and identification of the proteins from the 30S ribosomal subunit of Escherichia coli have been completed in several laboratories (Dzionara, Kaltschmidt and Wittmann, 1970; Kurland, Voynow, Hardy, Randall and Lutter, 1969; Traut, Moore, Delius, Noller and Tissieres, 1969; Nomura, Mizushima, Ozaki, Traub and Lowry, 1969; Sypherd, O’Neil and Taylor, 1969). Direct comparisons of the 30S proteins obtained from the different laboratories have led to a uniform nomenclature for these proteins (Wittmann et al., 1971). The analysis of the proteins from the larger 50S subunit has not progressed as well.

Electrophoretic fractionation of the proteins from the 50S subunit of Escherichia coli has suggested that thirty-four proteins could be obtained from this particle (Traut et al., 1969; Kaltschmidt and Wittmann, 1970). In addition, amino acid compositions and molecular weights have been reported for many of the 50S proteins (Traut et al., 1969; Dzionara et al., 1970; Kaltschmidt et al., 1970). However, earlier chemical analysis of the proteins obtained from the 30S subunit has shown that a single chemical species can give rise to multiple electrophoretic forms (Hardy et al., 1969; Craven et al., 1969). Thus, aggregation, oxidation, enzymatic cleavage or chemical modification by degradation products of urea have been identified as potential sources of such electrophoretic artifacts. The physical characteristics and amino acid compositions of the proteins do not necessarily reveal all such artifacts. Therefore, evidence that the protein has a distinct amino acid sequence seems to be desirable for the identification of an electrophoretic component as a unique chemical species.

Similarly, the electrophoretic homogeneity of a sample is not an unambiguous measure of chemical homogeneity. For this reason, data that can be used to determine the number of chemical species in a sample are required to establish the
degree of purity of the proteins obtained from ribosomes (Hardy et al., 1969; Craven et al., 1969). Thus, the elegant two dimensional electrophoretic fractionation of the ribosomal proteins depends on a prior chemical characterization of these proteins for its validity as a definitive measure of the number of proteins present in the ribosomes (Kaltschmidt and Wittmann, 1970).

Accordingly, we have characterized the proteins of the 50S subunit with respect to tryptic peptides, amino acid compositions and molecular weights, as well as by their electrophoretic characteristics. Twenty-seven proteins have been purified and so characterized. A number of additional electrophoretic components have been isolated, but these seem to be artifacts. The mass of the 50S subunit is large enough to accommodate one copy of all twenty-seven 50S proteins.

2. Methods

Ribosomes were prepared from Escherichia coli B as described previously (Kurland, 1966; Hardy et al., 1969). The 50S subunit were prepared by zonal centrifugation as described by Hardy et al. (1969). However, in order to ensure the purity of these preparations, the crude 50S subunits were run a second time in the zonal centrifuge to minimize 30S subunit contamination.

Extraction of proteins was done by the modified acetic acid method described by Hardy et al. (1969).

Chromatography on cellulose phosphate and Sephadex was performed as described by Hardy et al. (1969). Chromatography on DEAE cellulose (0.95 meq/gm, Sigma Chemical Co.) columns was also employed with some of the acidic 50S proteins. The DEAE-cellulose was cleaned and regenerated in a way similar to that employed with the cellulose-phosphate. 10–12 mg of protein in Tris-Urea buffer (0.01 M Tris, 0.02 M NaC1, 61V[ Urea 0.12 M methylamine, 0.01 % β-mercaptoethanol, pH 8.5) were chromatographed on columns (1.4 × 20cm) of DEAE-cellulose. Such columns were developed with 300 or 400 ml linear gradients of NaCl (0.02 M to 0.15 M). Finally, CM-cellulose (0.54 meq/gm, Biorad Laboratories) was also used for chromatography in the same way as for DEAE-cellulose. However, here the buffer was made up with acetate and urea (0.005 M Na acetate, 0.01 M NaCl, 6 M urea, 0.012 M methylamine, 0.01% β-mercaptoethanol, pH 5.6).

Alkylation of the 50S proteins was routinely employed to prevent the aggregation of the proteins through disulfide bonds. Solid Tris was added to bring the protein solution (1 mg/ml) to pH 8.3. Then, iodoacetamide was added to a final concentration of 0.01 M. After incubating the solution for 20 min at 37°, the reaction was stopped by the addition of β-mercaptoethanol to a final concentration of 0.05 M. The proteins were exhaustively dialyzed against standard buffer, pH 6.5 (6 M Urea, 0.05 M NaH2PO4, 0.012 M methylamine, 0.01% β-mercaptoethanol) before they were fractionated.

Ammonium sulfate precipitation of the 50S proteins was employed to obtain group fractionation of the proteins prior to chromatography. All of the precipitations and centrifugations were done in the cold (0–2°). The starting solution in standard buffer, pH 6.5 contained approximately 1 mg/ml of protein.

176 g/liter of ammonium sulfate are added slowly with stirring. The suspension is stirred for 30 min and then centrifuged for 30 min at 13000 × g. There is rarely any precipitate at this stage. The supernatant is brought to pH 6.5 and 94 g of ammonium sulfate are added per liter as before. After stirring and centrifuging the mixture (here and subsequently all stirring and centrifugation is as described for the first step), a pellet and supernatant are recovered. The pellet fraction dissolved in standard buffer, pH 6.5 is fraction A.

The supernatant is treated with another 65 g per liter of ammonium sulfate and resulting pellet forms fraction B. The supernatant fraction from this step is treated with 67 g/l of ammonium sulfate. The supernatant and pellet fractions are collected as above; the pellet is fraction C. Finally, this supernatant is treated with 70 g per liter of ammonium sulfate. The resulting pellet is fraction D and the supernatant is fraction E. Fractions A through E are exhaustively dialyzed before they are further fractionated by chromatography.