Correlation between 30S Ribosomal Proteins of Bacillus stearothermophilus and Escherichia coli

Katsumi Isono, Setsuko Isono, and Georg Stöffler
Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Germany*
Louis P. Visentin, Makoto Yaguchi, and Alastair T. Matheson
Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada**

Received October 3, 1973

Summary. The 30S ribosomal proteins from Bacillus stearothermophilus strains 799 and 10 were purified and correlated with those from E. coli by comparing their two-dimensional electrophoretic mobility, immunological cross-reaction, molecular weight, amino acid composition and partial amino acid sequence. A high degree of similarity was observed among the proteins from these taxonomically distant bacterial species.

1. Introduction

The ribosomes of Bacillus stearothermophilus, by virtue of their heat stability and higher optimal temperature for their function seem distinctly different from those of Escherichia coli. Indeed it was shown by Lodish (1969) that the ribosomes from these taxonomically distant organisms exhibit some unique properties in connection with the translation of RNA phage cistrons. However, early reconstitution experiments (Nomura et al., 1968) indicated an exchangeability of the ribosomal RNA and proteins of E. coli and B. stearothermophilus which yielded functionally active 30S hybrids. Furthermore, recent evidence indicates functional and structural correspondence between the individual 30S ribosomal proteins from these prokaryotes (Higo et al., 1973; Yaguehi et al., 1973).

In order to obtain further information on the common structural features which are shared by the ribosomal proteins from these two bacteria, we have purified the 30S ribosomal proteins from B. stearothermophilus strain 799 and from strain 10 to near homogeneity and correlated them with E. coli 30S proteins by comparing their molecular weight, amino acid composition, two-dimensional electrophoretic mobility, immunological cross-reaction and partial amino acid sequence. We present here evidences that ribosomal proteins from B. stearothermophilus are structurally very similar to those from E. coli.

2. Materials and Methods

a) Bacterial Strains. B. stearothermophilus strain 799 was used in Berlin and strain 10 in Ottawa throughout these experiments.
b) Purification and Characterization. Methods for purification of ribosomes and ribosomal proteins, molecular weight determination, amino acid composition analysis and partial amino

* Paper No. 82 on “Ribosomal proteins” — preceding paper is by J. Horne and V. A. Erdmann, FEBS Letters, in press.
** N.R.C.C. No. 13514.
acid sequence analysis will be described elsewhere (S. Isono and K. Isono, manuscript in preparation; L. Visentin, W. R. Rowsome, A. T. Matheson, and M. Yaguchi, manuscript in preparation; M. Yaguchi, C. Roy, A. T. Matheson, and L. P. Visentin, manuscript in preparation).

c) Two-Dimensional Electrophoresis on polyacrylamide gel was done as reported by Kaltenschmidt and Wittmann (1970) or with some modifications using a smaller chamber with the size of gel slabs of $10 \times 10 \times 0.3$ cm.

d) Immunological Cross-Reaction. Ouchterlony double diffusion tests to examine cross-reaction among the purified proteins from *B. stearothermophilus* and the antisera against *E. coli* 30S proteins were performed as previously described by Stöffler and Wittmann (1971).

e) Nomenclature. Proteins from *E. coli* are designated as E-S1, E-S2 etc. and those from *B. stearothermophilus* as B-S1, B-S2 etc.

3. Results and Discussion

As shown in Fig. 1, the two-dimensional electrophoretic patterns of 30s proteins from both *B. stearothermophilus* strains are basically very similar. However, some minor differences were observed between them. There are two protein spots, B-S1 and B-S9, in strain 10 (Fig. 1 b) which appear to be very faint or absent in strain 799 (Fig. 1 a). B-S1 resembles E-S1 in molecular weight, mobility on two-dimensional acrylamide gels and in amino acid composition. B-S9 appears to correspond to E-S6. Differences in mobility of certain proteins have also been observed. B-S16 and B-S17 migrate slightly faster while B-S6 migrates slightly slower in the first dimension in strain 10 than in strain 799. These may be strain specific proteins analogous to E-S5 and E-S7 in *E. coli*.

We have also examined the amino acid composition, immunological cross-reaction, molecular weight, two-dimensional electrophoretic mobility and partial amino acid sequence of the individual purified proteins and thus have correlated them with *E. coli* proteins. As presented in Table 1, 14 out of the 20 purified proteins from strain 799 were found to cross-react with the antisera against *E. coli* 30s proteins. Of these, B-S11, B-S14, B-S17 and B-S18 showed strongest cross-reaction with the antisera against *E. coli* E-S11, E-S13, E-S19 and E-S15, respectively. A protein, located between B-S1 and B-S2, cross-reacted with anti-E-S8 serum. The amino acid composition of this protein was also very similar to that of E-S8, although its molecular weight (27000 daltons) was found to be much larger than that of the latter (15500).

Striking sequence similarities were found in 15 of the 20 purified proteins from strain 10 and *E. coli* by determining the amino acid sequence of the first 12 residues from the N-terminal position. A summary of the sequence similarities is given in Table 1. In the case of B-S4, B-S5, B-S8, B-S10, B-S12, B-S14, B-S17, and B-S20 and the corresponding *E. coli* proteins, at least 8 out of the first 12 residues were found to be in the same sequence. In the case of B-S1, B-S6, and B-S19 no comparison of sequence homologies with the equivalent *E. coli* proteins could be obtained since B-S1, E-S5, and E-S18 have blocked N-terminal groups.

The correlation by amino acid sequence analysis of the proteins from strain 10 and *E. coli* are in complete agreement with the immunological correlation established for the proteins from strain 799 and *E. coli*, except for B-S2, B-S3, B-S4 and B-S9 (see Table 1). From these data, we can conclude that some of the ribosomal proteins (for examples, E-S11, E-S13, E-S15, and E-S19) are very