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Amino-Terminal Sequences of Some *Escherichia coli* 30S Ribosomal Proteins and Functionally Corresponding *Bacillus stearothermophilus* Ribosomal Proteins[1]

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Amino-terminal sequences of five purified *Escherichia coli* 30S ribosomal proteins (S4, S9, S10, S16, and S20) were compared with those of their functionally corresponding *Bacillus stearothermophilus* ribosomal proteins identified previously by the reconstitution technique. An automatic Edman degradation method was used for sequence determinations. The sequence of the first 30 residues is presented, except that only the first 25 residues are shown for the S20 pair. Substantial (40 to 70%) sequence homologies have been observed in every case. The results show that the pairs of functionally equivalent proteins, previously identified by the reconstitution technique, are also chemically related. Thus, the present chemical studies give further support for the previous conclusion that two ribosomes with different properties, 30S subunits from *E. coli* and *B. stearothermophilus*, have the same fundamental structural organization.

Earlier experiments from this laboratory demonstrated that functionally active 30S ribosomal subunits can be reconstituted from the 16S ribosomal ribonucleic acid (rRNA) of one species of bacteria and that the ribosomal proteins (r-proteins) can be reconstituted from a distantly related species (18). It has been suggested that the specific part of r-proteins interacting with the rRNA may have a structural feature in common with "corresponding proteins" from different bacterial species. In extending this work further, we have recently performed experiments to determine whether r-proteins from distantly related bacterial species can be shown to be functionally equivalent on a one-to-one basis (11). For this purpose, we fractionated 30S r-proteins from *Bacillus stearothermophilus* (*B* proteins) and looked for functional correspondence between these proteins and *Escherichia coli* 30S r-proteins (*E* proteins) by using the reconstitution technique. The properties of the ribosomes from these two organisms are different in several respects, as discussed in previous papers (11, 18). There are distinct chemical differences between the 16S RNAs of the two species (18, 21 and papers cited therein). Moreover, the proteins from 30S subunits differ with respect to their column chromatographic (11) or gel electrophoretic patterns (1, 18, 26), their ability to confer heat resistance upon the ribosomal subunits (9, 18, 20), and their immunochemical properties (29). In addition, it is known that *B. stearothermophilus* 30S subunits cannot translate the coat protein and the replicase cistrons of RNA messenger from f2 and related RNA phages, whereas *E. coli* 30S subunits can (15). However, we have found that most, if not all, of the *E* proteins have functionally equivalent counterparts among *B* proteins, supporting the conclusion that the fundamental structural organization of ribosomes is the same throughout prokaryotic organisms (11).

Complete interchangeability between *E* proteins and *B* proteins in the 30S reconstitution was somewhat surprising in view of the known differences between the ribosomal proteins of the two species described above, and especially surprising in view of the results of the immunochemical studies which showed very weak cross-reaction between *E* and *B* ribosomal proteins (29). In addition, our previous conclusion on the conservation of ribosome structure, as well as r-proteins, depended for the most part on the functional assay of proteins by the reconstitution technique (11). Since we cannot completely exclude fortuitous stimulation of activity of protein-deficient reconstituted "30S" particles by some unrelated proteins, rigorous proof of

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one-to-one correspondence between *E* proteins and *B* proteins must await complete purification of all of the *B* proteins and comparison of their amino acid sequence with the sequence of the functionally corresponding *E* proteins. For this reason, we have started comparative sequence studies of *E* and *B* proteins.

Amino-terminal sequences of five purified *E* proteins (S4, S9, S10, S16, and S20) have now been compared with those of their functionally corresponding *B* proteins identified previously by the reconstitution experiments. The results presented below support the previous conclusion of one-to-one correspondence between *E* proteins and *B* proteins.

MATERIALS AND METHODS

E proteins were purified as described previously (10), except that *E. coli* strain MRE600 was used instead of strain Q13. *B. stearothermophilus* strain 799 was grown at 65 C in a complex medium as described previously (8). The methods for preparation of ribosomal subunits, ribosomal RNA, and total ribosomal protein mixtures from *B. stearothermophilus* have also been described (8). Many *B* proteins were purified by a phosphocellulose column chromatography at pH 8.0, followed by Sephadex G-100 column gel filtration as described (11). Some proteins were purified further by a second phosphocellulose column chromatography at pH 6.5. Details of the purification procedures of *B* proteins will be published elsewhere. Purity of the proteins was examined by two-dimensional polyacrylamide gel electrophoresis (12). Four of the five *B* proteins analyzed here showed a single spot; the protein corresponding to *E. coli* S9 was the single exception. This *Bacillus* protein (called *B* S9) showed one major spot and a second minor spot that was very close to the major spot on the gel plate. Since the sequence work showed only one amino-terminal sequence with a high homology to *E* S9 (see below), the second minor spot may be a derivative of *B* S9, such as an oxidized form of *B* S9, or else a contaminant with a blocked N-terminal amino acid.

Edman degradations were performed automatically (7) with a Beckman model 890 Sequencer. About 100 to 300 nmol of protein was dissolved in 88% formic acid containing ethanethiol (10%, vol/vol) and applied to the Sequencer, and the procedures described in the Beckman Sequencer manual were followed. Thiazolinone derivatives in butyl chloride extracts obtained after each degradation step were converted to phenylthiohydantoin (PTH) derivatives by heating in 1.0 N HCl at 80 C for 10 min, and the products were extracted with ethyl acetate.

A Beckman GC-45 gas chromatograph equipped with DC560 or SP400 as the supports was used to identify PTH amino acids directly and also after silylation with *N,O*-bis(trimethylsilyl)acetamide (J. J. Pisano and T. J. Bronzert, Fed. Proc. **29**:916, 1970; reference 22). In addition, several other methods were used to identify the PTH amino acids. Polyamide thin-layer chromatography was performed with solvents IV (carbon tetrachloride and glacial acetic acid [80:30, vol/vol]) and VI (30% acetic acid) as described by Kulbe (14). Solvent VI was particularly useful to confirm PTH derivatives of asparagine, aspartic acid, glutamine, and glutamic acid, provided there was enough material. Detection of PTH amino acids on thin-layer chromatograms was done with the iodine-azide reagent (6). At the early stage of this work, PTH-Arg and PTH-His were identified by the phenanthrenequinone reaction (31) and the Pauly reaction (5), respectively. For these reactions, PTH amino acid samples were first subjected to electrophoresis on cellulose thin-layer sheets (10 by 10 cm) in 0.02 M phosphate buffer (pH 6.2) at 650 V for about 8 min, and the reagents were then applied (cf. reference 6).

In later experiments, PTH amino acids were also identified as amino acids after hydrolysis with HI as described by Smithies et al. (24). This method not only gave unambiguous identification of PTH-Arg and PTH-His, but also was useful in confirming the conclusions obtained by the gas chromatographic method. Amino acid analysis of the hydrolyzates was done with a JEOL 6AH amino acid analyzer.

To facilitate the identification of cysteine residues, S4 and S20 from *E. coli* were reduced and carboxymethylated in 8 M urea with [^{14}C]iodoacetate essentially as described by Slobin and Singer (23). Portions of samples from each step of automatic Edman degradation were then analyzed for ^{14}C in a Packard Tri-Carb liquid scintillation counter.

RESULTS

B and E proteins studied for sequence comparison. Five purified *E* proteins (S4, S9, S10, S16, and S20) and their functionally corresponding proteins (*B* S4, *B* S9, *B* S10, *B* S16, and *B* S20, respectively) were compared with respect to their amino-terminal amino acid sequences. Although the correspondence of *B* S9 to *E. coli* S9 (*E* S9) and of *B* S20 to *E* S20 was first deduced by immunochemical cross-reaction with specific antisera (11), later reconstitution assays using the standard method have established that the hybrid 30*S* subunits containing *B* S9 instead of *E* S9, or *B* S20 instead of *E* S20, have the same activity as the control particles in poly[U]-dependent polyphenylalanine synthesis which is higher than that of S9- or S20-deficient particles (unpublished experiments). The other three *B* proteins were originally detected by their ability to replace specifically one of the *E* proteins in the reconstitution assay. They did not show immunochemical cross-reaction with functionally corresponding *E* proteins under the conditions we used. All five *B* proteins were purified to homogeneity (for *B* S9, see above), and their amino-terminal amino acid sequences were determined. The corresponding five pure *E* proteins were simi-

larly examined for their amino-terminal sequences.

Amino-terminal amino acid sequence analysis. The methods for sequence determinations are described above. Automatic sequence determinations could be done for 25 to 60 residues. We show the results for the first 30 residues, except in the case of S20 where only the first 25 residues are shown. At least two determinations were done using different preparations for each protein.

Table 1 shows the results of the methods actually used to identify PTH amino acids obtained from each step, and the deduced sequences for *E* S4 and *B* S4. In this example, all the first 30 amino acid residues were unambiguously identified.

Figure 1 shows the sequence results obtained

TABLE 1. *Sequential degradation of E. coli and B. stearothermophilus S4 proteins*[a]

Step no.	*E. coli* S4							*B. stearothermophilis* S4			
	Deduced residue[b]	GC[c]		TLC[e]	Staining		AAA[g]	Deduced residue[h]	GC[c]		AAA[g]
		−S[d]	+S[d]		Pauly	PNQ[f]			−S[d]	+S[d]	
1	Ala	Ala	Ala					Ala	Ala	Ala	Ala
2	Arg					+	Arg	Arg			Arg
3	Tyr	Tyr	Tyr					Tyr	Tyr	Tyr	Tyr
4	Leu	Leu/Ile	Leu					Thr	Thr	Thr	Aab
5	Gly	Gly	Gly					Gly	Gly	Gly	Gly
6	Pro	Pro?	Pro?	Pro			Pro	Pro	Pro	Pro	Pro
7	Lys	Lys	Lys				Lys	Met	Met	Met	
8	Leu	Leu/Ile	Leu					Trp	Trp	Trp	
9	Lys	Lys	Lys	Lys			Lys	Lys	Lys	Lys	Lys
10	Leu	Leu/Ile	Leu				Leu	Ile	Leu/Ile	Ile	Ile
11	Ser	Ser/Cys		Ser?			Ala	Ser	Ser/Cys	Ser/Cys	Ala
12	Arg				−	+	Arg	Arg			Arg
13	Arg				−	+	Arg	Arg			Arg
14	Glu		Glu				Glu	Leu	Leu/Ile	Leu	
15	Gly	Gly					Gly	Gly	Gly	Gly	Gly
16	Thr	Pro/Thr		Thr			Aab	Ile	Leu/Ile	Ile	
17	Asp		Asp	Asp				Ser	Ser/Cys	Ser/Cys	Ala
18	Leu	Leu/Ile	Leu					Leu	Leu/Ile	Leu	
19	Phe	Phe	Phe					Ser	Ser/Cys	Ser/Cys	Ala
20	Leu	Leu/Ile	Leu				Leu	Gly	Gly	Gly	Gly
21	Lys		Lys				Lys	Thr	Pro/Thr	Pro/Thr	Aab
22	Ser	Ser/Cys					Ala	Gly	Gly	Gly	Gly
23	Gly	Gly	Gly				Gly	Lys	Lys	Lys	Lys
24	Val	Val	Val					Glu		Glu	Glu
25	Arg				−	+	Arg	Leu	Leu/Ile	Leu	Leu
26	Ala	Ala	Ala					Gln		Gln	Glu
27	Ile	Leu/Ile	Ile				Ile	Lys		Lys	Lys
28	Asp		Asp	Asp				Arg			Arg
29	Thr	Pro/Thr	Thr?				Aab	Pro	Pro	Pro	Pro
30	Lys		Lys				Lys	Tyr	Tyr	Tyr	Tyr

[a] Only the data for S4 are shown here as a typical example of our identification procedures. The results of two or more independent sequence determinations are combined for each protein.

[b] Assignment of Ser or Cys is based on analysis of samples from each step for ^{14}C.

[c] GC, Gas chromatography.

[d] +S, −S, PTH amino acids analyzed with (+S) and without (−S) silylation.

[e] TLC, Polyamide thin-layer chromatography.

[f] PNQ, Phenanthrenequinone reaction.

[g] AAA, Amino acid analysis of hydrolyzed PTH amino acids. The residues from S4 of *E. coli* were identified initially without use of the amino acid analyzer. Later several PTH amino acids were analyzed by amino acid analysis, and the initial identifications were confirmed. Some PTH amino acids do not give parental amino acids upon hydrolysis with HI; they were identified as follows (24): PTH-Thr—α-amino butyric acid (Aab); PTH-Ser, -*S*-carboxymethyl-Cys— alanine; PTH-Ile—isoleucine plus alloisoleucine (shown in the table as Ile).

[h] Assignment of Ser is based on the absence of cysteine in this protein (K. Higo and L. Kahan, unpublished results).

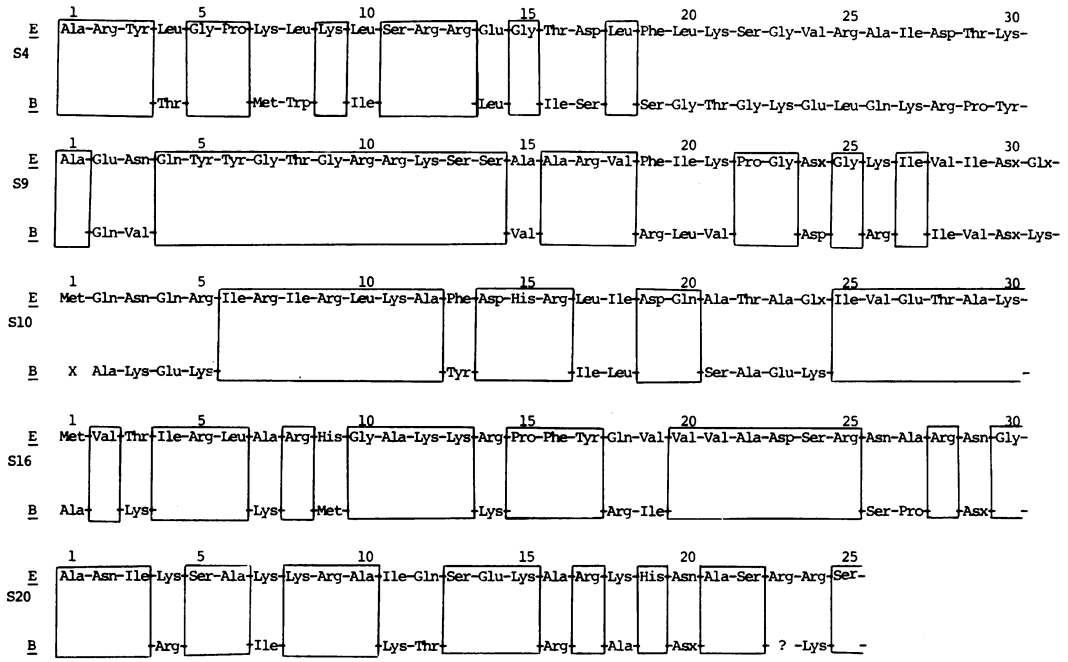

FIG. 1. *Comparison of partial amino acid sequences of five functionally corresponding pairs of r-proteins from Escherichia coli (E) and from Bacillus stearothermophilus (B). The residues are numbered according to the sequence of E. coli proteins. B. stearothermophilus residues are given where they differ from those in E. coli, but otherwise left blank. The regions showing homology are boxed. The "?" at position 23 of B S20 indicates an unidentified residue. Note that the first residue of B S10 is aligned with position 2 of the E S10 sequence. Asx and Glx indicate aspartic acid or asparagine and glutamic acid or glutamine, respectively.*

for the 10 proteins studied in this way. Sequences of two functionally corresponding *E* and *B* proteins are shown in parallel. In the case of S10, the first amino acid residue of the *B* protein is aligned with the second amino acid residue of the *E* protein. In several cases, distinction between Asp and Asn, or Glu and Gln, was not made. In addition, we failed to make definite identification of the 23rd residue of *B* S20.

DISCUSSION

It is clear that functionally corresponding proteins from the two organisms also have correspondence with respect to sequence similarity (Fig. 1). Thus, present results confirm the previous functional identification of one-to-one protein correspondence and give strong support to the conclusion that the fundamental structural organization of ribosomes is essentially the same throughout prokaryotic organisms. However, to complete rigorous proof of one-to-one correspondence, we are currently extending studies, similar to the one presented here, to other *E* and *B* proteins.

Table 2 summarizes the similarity in sequence between two proteins in each pair compared. Although the sequences compared represent only 14 to 25% of the entire amino acid residues of five protein pairs (cf. Table 2, columns 8 and 9), the presence of sequence homology is unambiguous. About 60 to 70% homology with respect to amino acid sequence was found with four protein pairs, and 37% homology was found with the protein S4 pair. In addition, many differences observed in the protein pairs S9 and S10, which occur between large blocks of homology, involve amino acid changes between chemically similar residues, such as Arg↔Lys, Ile↔Leu, Ile↔Val, Phe↔Tyr, and Ala↔Val. Thus, two of the protein pairs probably have similar conformations at the N-terminal region regardless of their species origin.

Many of the amino acid differences between the two proteins of these pairs can be explained by a single base change in the presumed nucleotide sequence of their structural genes (Table 2).

Owing to lack of available amino acid sequence data on "reference" proteins from these two organisms, it is at this time not clear

TABLE 2. *Summary of the amino acid sequence similarity between E. coli proteins and functionally equivalent B. stearothermophilus proteins*

Proteins compared	No. of amino acid residues compared	No. of amino acid residue differences[a] (%)	Amino acid differences explicable by			Total base change[b] (%)	Sequence compared/total no. of amino acids in protein[c]	Immunochemical cross-reaction[d]
			Single-base change	Two-base change	Three-base change			
B S4-*E* S4	30	19 (63%)	11	8	0	27 (30%)	30/209 (14%)	
B S9-*E* S9	31	10 (32%)	7	3	0	13 (14%)	31/132 (23%)	+
B S10-*E* S10	29	11 (38%)	10	1	0	12 (14%)	29/164 (18%)	
B S16-*E* S16	30	9 (30%)	6	2	1	13 (14%)	30/118 (25%)	
B S20-*E* S20	24	7 (29%)	4	3	0	10 (14%)	24/114 (21%)	+

[a] Asx24-Asp24 and Asx30-Asx30 in S9, Asn29-Asx29 in S16, and Asn20-Asx20 in S20 were not included.

[b] These values are "minimal number of base changes required." Glx-Lys at position 31 in S9 also at position 24 in S10 is explicable by a single-base change regardless of Glx being Glu or Gln.

[c] Total number of amino acids in proteins were estimated from the molecular weight of *E* proteins obtained by equilibrium sedimentation (4), assuming the average molecular weight of amino acid was 110. Molecular weights of *B* proteins are probably very similar to the molecular weights of corresponding *E* proteins (our unpublished experiments; see also reference 26).

[d] Higo et al. (11), and unpublished experiments.

whether ribosomal protein structures are, in general, more highly conserved than those of other cellular proteins. However, it may be of interest to note here that the results of deoxyribonucleic acid-RNA hybridization competition experiments suggest there is only about 20% base sequence homology between rRNAs from *E. coli* and *B. stearothermophilus* (21). Furthermore, dissimilarities of amino acid sequences of as much as 30% between species within the family *Enterobacteriaceae* have been inferred from the immunological comparisons of alkaline phosphatases and tryptophan synthetase alpha subunits (reference 2 and papers cited therein).

It would be interesting to extend the present sequence studies further, including several other bacterial species, and to find the stretches of invariable amino acid sequences among many functionally equivalent r-proteins with diverse origins. Such invariable regions must reflect structures important to the role of the pertinent protein in ribosome assembly or function. For example, some ribosomal proteins appear to be required only for assembly of the particles and not for function of the finished particles (16). In such proteins, the "conserved" regions may be the ones participating directly in interactions with 16*S* RNA or other proteins. In contrast to the close sequence similarity of the pairs of functionally equivalent proteins, no obvious N-terminal sequence similarities were found in comparisons of functionally unrelated *B* and *E* proteins.

In addition to the five pairs of proteins reported here (S4, S9, S10, S16, and S20), we have examined the amino-terminal sequences of 13 other *E* proteins, totaling 18 proteins analyzed out of 21 30*S* proteins from *E. coli* as well as two other *B* proteins (S12 and S19) analyzed, making a total of seven (K. Higo, B. Ballou, K. Loertscher, and A. Vassos, unpublished experiments). The same conclusions were reached. Two other functionally related *E-B* pairs (S12 and S19) showed very high sequence homologies, whereas no obvious homology has been found among the 18 different *E* proteins or the seven different *B* proteins so far examined. We have found, however, some partial sequence homologies among *E. coli* proteins. For example, the sequence from 16th to 27th position of *E. coli* S19 has a high homology to the sequence from 32nd to 43rd position of *E. coli*, S20, as shown in the following sequences:

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          16                                  27
S19:  Lys-Lys-Val-Glu-Lys-Ala-
                        Val-Glu-Ser-Gly-Asp-Lys

S20:  Lys-Lys-Val-Tyr-Ala-Ala-
                        Ile-Glu-Ala-Gly-Asp-Lys
          32                                  43
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The results are consistent with the previous conclusion that all of the 21 30*S* r-proteins are functionally and chemically different (3, 13, 17, 25, 27, 28). After completion of this work, we learned that Yaguchi et al. (30) determined the N-terminal sequences of three *B* proteins which are homologous to the sequences of *E. coli* S3, S9, and S13, respectively.

So far, we have emphasized the presence of a high degree of sequence homology between *E*

proteins and their counterparts which supports a notion of a common structural organization among diverse bacterial species. However, as stated above, there are several clear differences in properties between *E. coli* ribosomes and *B. stearothermophilus* ribosomes. For example, although amino acid sequences of *E* and *B* proteins have not as yet revealed any clue as to the heat stability difference between the two ribosomes, such sequence data may eventually become useful for understanding this problem. Another important difference between the two ribosomes is concerned with the specificity of initiation of translation of natural messenger RNA (see above). We have demonstrated recently that the 30*S* components responsible for this difference are mainly S12 and 16S rRNA; S12 has a unique role in the initiation of translation (19; W. Held, W. Gette, and M. Nomura, unpublished experiments). It would be a challenging problem to compare the amino acid sequence of *E* S12 with that of *B* S12 and to find a chemical basis for the initiation specificity difference between *E. coli* and *B. stearothermophilus* ribosomes.

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