Volume 331, number 1,2, 9–14 FEBS 12994 September 1993


# Isolation and amino acid sequence of the 30S ribosomal protein S19 from *Mycobacterium bovis* BCG

Naoya Ohara[a], Makoto Kimura[b], Yoshinori Higashi[b], Takeshi Yamada[a,*]

[a]*Nagasaki University, School of Dentistry, 1-7-1 Sakamoto-machi, Nagasaki 852, Japan*
[b]*Laboratory of Biochemistry, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan*

Received 19 July 1993

The 30S ribosomal proteins from *Mycobacterium bovis* BCG were separated by reverse phase-high performance liquid chromatography (RP-HPLC). The isolated proteins were analyzed by SDS-PAGE, blotted on PVDF-membranes and subjected to sequence analyses using a gas-phase sequencer to correlate them to those of the well studied *Escherichia coli* and *Bacillus stearothermophilus* ribosomes. Moreover, the internal amino acid sequence of one ribosomal protein, MboS19, which is homologous to *E. coli* ribosomal protein S19 (EcoS19) and *B. stearothermophilus* ribosomal protein S19 (BstS19), was further analyzed by sequencing its internal peptides and two segments from the N- and C-termini of the protein were selected to deduce the sequence of two oligonucleotide primers which were used in a polymerase chain reaction. Using the amplified DNA fragment thus obtained as a hybridization probe, the gene encoding protein S19 was identified and cloned. Sequence analysis of the DNA fragment, together with peptide sequence analysis could determine the complete amino acid sequence of MboS19. This sequence proved to be 64% and 71% identical to those of the corresponding S19 proteins from the eubacteria *E. coli*, and *B. stearothermophilus*, respectively; 33% of the residues of MboS19 were identical to those in the archaebacteral ribosomal protein HmaS19

*Mycobacterium bovis* BCG; Ribosomal protein; Amino acid sequence

## 1. INTRODUCTION

Biological and immunological studies on proteins from *Mycobacterium bovis* BCG (BCG) which provoke humoral and cellular immune responses have been stimulated by the fact that a considerable number of people, particularly in the developing countries continue to suffer from tuberculosis and that patients infected with a human immunodeficiency virus are readily infected with mycobacteria. In addition, it is hoped that these studies may lead to the development of an improved BCG vaccine to replace the one that has been widely used since 1948 and which gives a low incidence of serious complications.

Thus, a large number of *M. bovis* BCG proteins, including heat shock proteins [1,2] and secretory proteins [3–5], have been identified and investigated by biochemical and immunological methods. In addition to these proteins also ribosomal preparation from BCG has been shown to elicit the delayed hypersensitivity reaction in guinea pig immunized with ribosomal proteins [6–9]. The immunological importance of a ribosomal preparation from mycobacteria is also shown by the fact that guinea pigs and mice immunized with a ribosomal preparation from *Mycobacterium tuberculosis* $H_{37}Ra$ were protected from infection by pathogenic *M. tuberculosis* $H_{37}Rv$ [10]. The fact that a ribosomal preparation (ribosomal vaccine) is protective is accepted as a general principal for most microorganisms [11].

Furthermore, since mycobacteria grow slowly with generation times varying from several hours to more than ten days, the study on the structure and function of their ribosomes could be important for a better understanding of the mechanism responsible for slow growth.

Mycobacterial ribosomes possess unique and interesting properties: they were found to be unable to translate f2RNA in a cell-free system [12] and this feature was thought to be due not only to a possible incompatibility of S1 protein but also perhaps to more general differences in the structure of the ribosome.

To gain a better insight into the regulation of mycobacterial translation, the isolation and characterization of ribosomal components are essential requirements. Furthermore, when the mycobacteria rRNA gene organization [13] and sequence [14] were investigated, it was found that they had unusual features. Thus, although the nucleotide sequence of *M. bovis* BCG rRNA is 75% homologous to that of *E. coli*, the overall number (two) and organization of the rRNA genes resembles

*Corresponding author. Fax: (81) (958) 43 1060.

*Abbreviations:* MboS19, *Mycobacterium bovis* BCG ribosomal protein S19; EcoS19, *Escherichia coli* ribosomal protein S19; BstS19; *Bacillus stearothermophilus* ribosomal protein S19; RP-HPLC; reverse phase-high performance liquid chromatography; TFA, trifluoroacetic acid; PVDF, polyvinylidendifluoride.

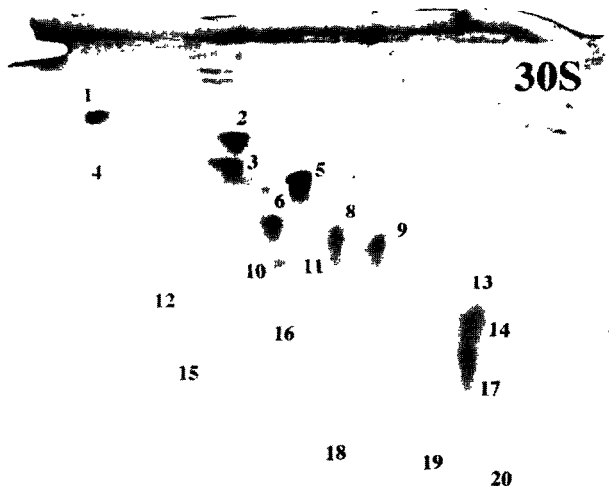



Fig. 1. Two-dimensional gel electrophoretograms of 30S ribosomal proteins from *M bovis* BCG

those of archaebacteria, such as *Halobacterium halobium* [15], *Thermus thermophilus* [16], and *Mycoplasma capricolum* [17]. In addition, at least two transcriptional initiation sites were observed in rRNA operon of BCG; the one was recognized by *E. coli* RNA polymerase in a cell-free system, whereas the other was not [22].

In contrast to information on BCG-rRNA, few studies on its ribosomal proteins have thus far been carried out. To extend this study, we have started to elucidate the primary structures of the 30S ribosomal proteins of *M. bovis* BCG. We plan to accomplish this task in a short period of time using a limited amount of ribosomes by a combination of protein and DNA sequence methods. As an initial step of this study, we tried to isolate ribosomal proteins by RP-HPLC and then analyze their N-terminal amino acid sequences by a gas-phase sequencer. In this paper, we present the isolation of 30S ribosomal proteins from *M. bovis* BCG and correlate these to those of the well studied *E. coli* and *B. stearothermophilus*. Furthermore, we have determined the complete amino acid sequence of a *M. bovis* BCG ribosomal protein, which is homologous to *E. coli* S19, by the protein and DNA sequencing methods.

## 2. MATERIALS AND METHODS

### 2.1 *Materials*

The preparation of ribosomes from BCG and the zonal separation of ribosomal subunits have been described previously [23]. The ribosomal proteins were extracted from the 30S subunits with 66% acetic acid in the presence of 0.1 M $MgCl_2$ as described in [24], dialyzed against 5% acetic acid, and then lyophilized.

### 2.2. *Separation of proteins*

The proteins thus extracted were separated by RP-HPLC with a Hitachi L-6000 HPLC system. The protein mixture (2 mg of 30S total protein) was injected into a $C_4$ column (particle diameter, 5 $\mu$m; pore size 300 Å, column size, 4.6 × 250 mm; Yamamura Chemical Lab.) equilibrated with aqueous 0.1% TFA and chromatographed using a linear gradient of acetonitrile from 24 to 56% in 0.1% TFA over 90 min at flow rate of 0.6 ml/min. The eluate was monitored by measuring the absorbance at 220 nm. Proteins were manually collected and analyzed by SDS-PAGE as described below.

### 2.3. *Electrophoresis and electroblotting*

Two-dimensional electrophoresis of 30S ribosomal proteins was done as described in [25]. Proteins were analyzed by SDS-PAGE using 17.5% acrylamide gel [26] and then electrophoretically transferred to PVDF-membrane (Trans-Blot Transfer Medium, Bio-Rad) for 2 h at 180 mA on the ice. After transfer, the membrane was stained with 1% Ponceau S (Sigma Chemical Co.) in 1% acetic acid for one minute and destained in 1% acetic acid.

### 2.4 *Protein sequence analysis*

The protein MboS19 was digested with *Staphylococcus aureus* V8 protease (ICN ImmunoBiologicals) in 0.2 M *N*-methylmorpholine acetate buffer, pH 8.1, at 37°C for 18 h using an enzyme/protein weight ratio of 1:30. The mixture of peptides was separated by RP-HPLC on a column YMC GEL $C_4$ 300 Å, as described above for the separation of ribosomal proteins, except that the chromatography was

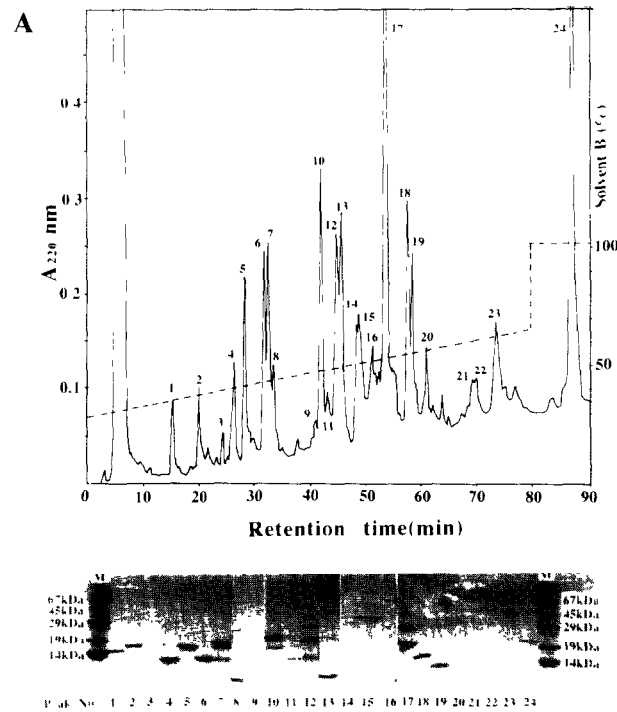



Fig. 2. Separation of 30S ribosomal proteins from *M bovis* BCG on a YMC-GEL C4 column (4.6 × 250 mm, 120 Å) and SDS-PAGE of individual fractions. (A) Separation of 30S total proteins dissolved in 30% acetic acid was done on a column equilibrated with 24% acetonitrile in 0.1% TFA, and eluted by a linear gradient of acetonitrile concentration from 24 to 56% at 0.6 ml/min at room temperature. (B) SDS-PAGE was done according to the method of Laemmli using a 17.5% acrylamide gel. Lane M indicates protein markers: bovine serum albumin (67 kDa), ovalbumin (45 kDa), carbonic anhydrase (45 kDa), myoglobin (19 kDa), and lysozyme (14 kDa).

done with a linear gradient of acetronile from 0 to 42% over 30 min. The amino acid was sequenced by a gas-phase sequencer PSQ-1(Shimazu). The reagents used for sequencing the protein were purchased from Wako Pure Chemicals.

2.5. *Cloning and DNA sequence analysis*

Procedures for the isolation and preparation of DNA from cultured BCG cells have been described previously [13]. On the basis of the amino acid sequences of the protein MboS19, the two mixed oligonucleotide primers with opposing orientations were synthesized by the solid-phase phosphoamidite method. The forward (5'-GGNCCNTTYGTNGAYGAR-C-3', where N = A,C,G, or T. Y = C or T and R = A or G) and the reverse (5'-CKNGTN-GGNGCRAA-YTCNCC-3', where K = G or T) primers were made for the sequences, GPFVDEH and GEFAPTR, respectively. The polymerase chain reaction (PCR) with Tth polymerase (Toyobo Co., LTD., Osaka, Japan) was done as recommended by the manufacturer. 1 ng of bacterial DNA was subjected to PCR in a total volume of 100 $\mu$l, with 2 U of Tth polymerase, 10 mM Tris-HCl (pH 8 9), 80 mM KCl, 1.5 mM $MgCl_2$, 0.05% (wt/vol) BSA, 0.1% Triton X-100, 0.1% sodium cholate, 50 pmol of each of the two primers, 1 mM each of deoxynucleotide triphosphates. The 100 $\mu$l mixture was covered by 100 $\mu$l of light mineral oil. The reaction was performed in an automated thermal Sequencer (Iwaki GlAss Co., LTD., Tokyo, Japan). The samples were first denatured by heating at 94°C for 3 min and then incubated for 35 cycles at 94°C for 1 min, 50°C for 2 min, and 72°C for 2 min. The samples were finally incubated for 5 min at 72°C. The amplified product was analyzed by electrophoresis on ethidium bromide-containing 2% agarose gels, and the DNA was visualized by transillumination The amplified product was blunted with T4 DNA polymerase and introduced into the *Sma*I site of the pUC18 plasmid. The nucleotide sequences of the DNA were determined by the dideoxy chain termination method [27].

Southern hybridization and labeling of the probe using the ECL direct nucleic acid labelling system (Amersham Japan Co., Ltd.) was done as described in the manufacturer's instructions.

Cloning of the 8-kb *Bam*HI fragments was done as follows. *M. bovis* BCG chromosomal DNA was digested with *Bam*HI and size-fractionated on 0.8% agarose. The DNA fragment of 8-kb in length, which gave a clear hybridized band with the probe, was cloned into the *Bam*HI site of pUC18. The ligated product was used to transformed *E. coli* JM109. The clones were screened by the colony hybridization as follows. The filters were prehybridized for 6 h in a solution of 5 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M Na-citrate), 5 × Denhard's solution, 0.1% SDS, and 125 $\mu$m/ml calf thymus DNA at 65°C. The hybridization was done at 58°C for 18 h in the same solution to which was added the nick-translated DNA. Possible possitive clones were amplified and again analyzed by Southern hybridization in the same way as described above.

## 3. RESULTS

### 3.1. *Separation of 30S ribosomal proteins*

Extraction of proteins from the 30S ribosomal subunits with acetic acid resulted in 18 protein spots on the two-dimensional electrophoretogram (Fig. 1). In order to determine the N-terminal amino acid sequence and correlate these proteins with those of the well studied eubacterial ribosomes, the proteins were separated by RP-HPLC and their N-terminal sequences were analyzed. The protein mixture extracted with acetic acid was applied to an RP-HPLC column YMC- GEL $C_4$ 300 Å (2.6 × 250 mm) equilibrated with 24% acetonitrile in 0.1% aqueous TFA, and then the proteins were eluted with a linear gradient from 24 to 56% acetonitrile concentration. Fig. 2 illustrates a typical separation of ribosomal proteins isolated from 30S ribosomal subunits prepared from *M. bovis* BCG. Approximately 24 peaks can be reproducibly detected in this chromatography. The analysis, by SDS-PAGE (Fig. 2), of the proteins present in the peaks indicates that in some cases, such as peak fractions 1, 2, 4, 5, 6, 23, and 24, the peaks appear to contain a single protein, while other peak fractions include several proteins. The proteins separated by SDS-PAGE were electrophoretically blotted onto a PVDF- membrane and subjected to sequence analysis with the aid of a gas- phase sequencer. Table I shows the N-terminal sequences of thirteen 30S ribosomal proteins and their homologues from *E. coli* and *B. stearothermophilus* searched by the computer program BLAST [28]. This analysis allowed us to correlate ten BCG ribosomal proteins with their corresponding eubacterial proteins. The identical amino acid residues among the homologous proteins is between 27 and 100%, although only four amino acid could be compared in the case of the protein in peak no. 4. These ten ribosomal proteins from BCG were designated according to their amino acid sequence homology to the corresponding *E. coli* proteins. The proteins homologous to *E. coli* S14 (EcoS14) and S12 (EcoS12) were referred to as MboS14 and MboS12, respectively. Although the N-terminal analysis of the proteins eluted in peak fractions, 5, 7, and 24 gave unambiguously 15 amino acid residues, their homologous proteins in the eubacterial ribosomes were not identified, suggesting that the primary structures of these proteins evolved in a highly divergent manner. Sequence analysis of proteins eluted at the other peaks gave no PTH amino acids, but no further attempt was made to obtain their sequences.

Table 1. The N-terminal sequences of 30S ribosomal proteins from Mycobacterium bovis BCG and their eubacterial homologues

| Peak No. | N-terminal sequence | Homologue | Identity (%) | |
|---|---|---|---|---|
| | | | Eco | Bst. |
| 1 | A K X S K I V K N Q R R A X T | S14 | 27 | 40 |
| 2 | P T I Q Q L V R K G R R D K I | S12 | 53 | 73 |
| 4 | A N I K S Q Q K R N R T N E R | S20 | 53 | 60 |
| 5. | P A K N V P A N S R R K A K H | | | |
| 6 | P R S L K K G P F V D E H L L | S19 | 87 | 80 |
| 7 | A E A K T G A K A A P R V A | | | |
| 10. | A V K I K L T R L G K I R N P | S16 | 40 | 53 |
| 13 | A L T A E Q I I E I L A S Y G | S15 | 27 | 40 |
| 17 | A R Y T | S4 | 75 | 100 |
| 18. | T M T D P I A A F | S8 | 56 | 56 |
| 19. | M R P Y E I M V V D A P | S6 | 50 | 56 |
| 23 | A V Y T M K Q L K D S G T H F | S2 | 33 | 60 |
| 24 | T E Y E G P K T K F H A L M Q | | | |

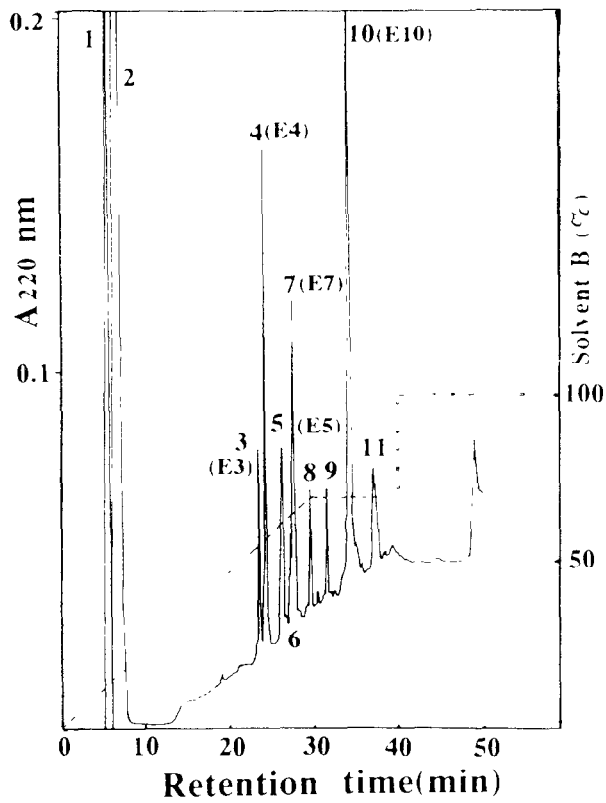



Fig. 3. Separation of peptides derived from *S aureus* V8 protease digestion of *M bovis* ribosomal protein S19. Separation was done on a YMC-GEL $C_4$ column (4.6 × 250 mm, 300 Å), and the peptides were eluted by a linear gradient of acetonitrile concentration from 0 to 42% in 0 1% TFA at a flow rate of 0.6 ml/min.

### 3.2. *Amino acid sequence of protein MboS19*

To obtain internal amino acid sequence information, MboS19 was digested with *S. aureus* V8 protease and the resulting peptides were separated by RP-HPLC (Fig. 3). This chromatography yielded eleven peaks, which were directly sequenced by a gas-phase sequencer. The sequence results of peptides E3 and E10, together with the N-terminal sequence analysis of the protein allowed us to extend the amino acid sequence from the N-terminus to residue $Ile^{30}$. Amino acid sequence analysis of peptide E7 provided the overlap of peptide E4 and E5, and localized it at the C-terminal region of the protein based on its sequence homology to those of EcoS19 and BstS19. In this peptide analysis, we could not obtain sequence information on the middle region and C-terminus of MboS19. Then, we selected two segments, G-P-F-V-D-E-H and G-E-F-A-P-T-R, for synthesizing two mixed oligonucleotide primers.

### 3.3. *Cloning and nucleotide sequence of the MboS19 gene*

The amplification reaction was carried out as described in section 2, using two primers based on the amino acid sequences described above, and the amplified gene was detected by agarose gel electrophoresis. In this experiment, the 200 bp fragment was specifically produced, and the fragment was treated with T4 DNA polymerase to make a blunt end. The amplified gene thus treated was then introduced into the *Sma*I site of the pUC18 plasmid and verified by DNA sequencing. DNA sequence analysis by the chain termination method revealed that the clone has an insert of 203 bp with part of the coding sequence of MboS19, beginning with GGCCG for Pro at position 6 and ending with GCT for Ala at position 74 of the protein (Fig. 4). Furthermore, the amino acid sequence deduced from the nucleotide sequence in the PCR product could align the *S. aureus* V8 protease peptides, as shown in Fig. 4. In this analysis, however, the C-terminus of the protein was not assigned unambiguously.

In order to clone a larger fragment containing the entire MboS19 gene, as well as other ribosomal protein genes, *M. bovis* BCG genomic DNA was digested with

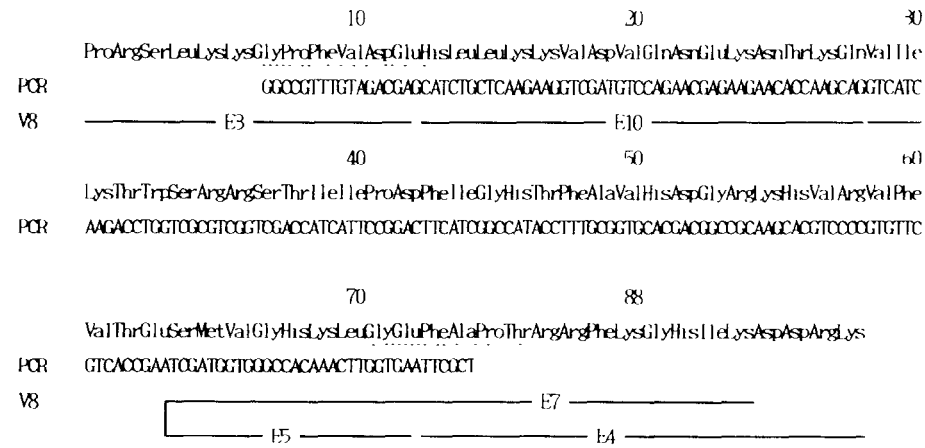
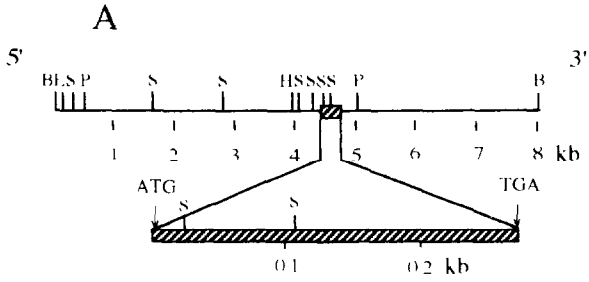



Fig. 4. The amino acid sequence of ribosomal protein S19 from *M bovis* BCG. The sequence was deduced by protein sequence analyses of the N-terminal amino acids and *S aureus* V8 protease peptides shown by solid lines, and also by sequencing the nucleotides of the amplified DNA fragment indicated by PCR. The dotted lines indicate the amino acid sequences used for the design of oligonucleotide primers.




```
                                                                  30
ATG CCA CGC AGC CTG AAG AAG GGG CCG TTT
M   P   R   S   L   K   K   G   P   F
                                                                  60
GTC GAC GAG CAT CTG CTC AAG AAG GTC GAT
V   D   E   H   L   L   K   K   V   D
                                                                  90
GTC CAG AAC GAG AAG AAC ACC AAG CAG GTC
V   Q   N   E   K   N   T   K   Q   V
                                                                 120
ATC AAG ACC TGG TCG CGT CGG TCG ACC ATC
I   K   T   W   S   R   R   S   T   I
                                                                 150
ATT CCG GAC TTC ATC GGC CAT ACC TTT GCG
I   P   D   F   I   G   H   T   F   A
                                                                 180
GTG CAC GAC GGC CGC AAG CAC GTC CCC GTG
V   H   D   G   R   K   H   V   P   V
                                                                 210
TTC GTC ACC GAA TCG ATG GTG GGC CAC AAA
F   V   T   E   S   M   V   G   H   K
                                                                 240
CTT GGT GAG TTC GCG CCG ACA CGC AGG TTC
L   G   E   F   A   P   T   R   R   F
                                                                 270
AAG GGC CAC ATT AAA GAC GAC CGA AAG AGC
K   G   H   I   K   D   D   R   K   S

AAG CGG CGA TGA
K   R   R
```

Fig. 5 Nucleotide sequence of the ribosomal protein S19 gene from *M bovis* BCG and the derived amino acid sequence. (A) A restriction map of the 8-kb *Bam*HI fragment containing the MboS19 gene. The coding region of the protein is illustrated by the hatched box. (B) The nucleotide and amino acid sequences of MboS19.

various restriction endonucleases and analyzed by Southern hybridization using the DNA fragment generated from the PCR as a hybridization probe. Since the 8-kb *Bam*HI fragments gave a hybridization signal in this analysis, a partial genomic library was made by ligation of the 8-kb *Bam*HI fragments of *M. bovis* BCG chromosomal DNA into the pUC 18 vector. The PCR product was nick-translated and colony-hybridized to the library of the 8-kb fragments of *M. bovis* BCG genomic DNA under the stringent condition as described above. The positive clone containing the 8-kb insert was selected for further study.

To localize the MboS19 gene on the 8-kb *Bam*HI insert, a restriction map was prepared by probing blots of the insert DNA digests. It was found from this analysis that the MboS19 gene locates in the middle region of the insert, and its nucleotide sequence was determined by the dideoxy chain termination method, as shown in Fig. 5. It should be noted that the nucleotide sequence of the MboS19 structural gene differs in three positions from that determined in the PCR product. The sequence analysis of the PCR product identified A, A, and T in place of C, G, and G, respectively, at positions 33, 219, and 225 in the gene.

The amino acid sequence deduced from the nucleotide sequence was coincident with those determined by amino acid sequencing of the amino-terminal region and the peptide fragments obtained by digestion with *S. aureus* V8 protease. The amino acid sequence of MboS19 thus deduced was aligned with bacterial homologous sequences, EcoS19 (29), BstS19 (30,31), and HmaS19 (32) (Fig. 6). Note that the N-terminal 39 amino acid residues in the protein HmaS19 are not included in this comparison. This comparison shows that MboS19 can easily be aligned with the two eubacterial S19 proteins with no insertions and deletions, and that MboS19 has 64 and 71% identical residues with EcoS19 and BstS19, respectively. In contrast, MboS19 shares only 33% identical residues with halophilic archaebacterial ribosomal protein HmaS19. Thus, proteins MboS19 from *Mycobacterium* is more related to the corresponding protein in the Gram-positive *B. stearothermophilus* than to that in the Gram-negative *E. coli*.

```
MboS19   P R S L K K G P F V D E H I L   K K V D V Q N E K N I K Q V I   30
EcoS19   P R S L K K G P F I D L H L L   K K V F K A V E S G D K K P L   30
BstS19   G R S L K K G P F S D E H L M   K K I F K I N E T G Q K Q V I   30
HmaS19   R R S I V R G I T E E K H K L I E K A R E A G E E E I A N D P I   71

MboS19   K T W S R R S I I I P D F I G H T F A V H D G R K H V R V F V T   62
EcoS19   R T W S R R S T I F P N M I G L T I A V H N G R Q H V P V F V T   62
BstS19   K T W S R R S T I F P Q F V G H T I A V Y D G R R H V P V Y I T   62
HmaS19   R T H L R D M P V V P E M V G L T L A V H D G Q N F E R V K V E   103

MboS19   F S M V G H K I G E F A P T R R F K G H I K D D R K S K R R   92
EcoS19   D E M V G H K L G F F A P T R T Y R G H A A N K K A K K K   91
BstS19   F D M V G H K L G E F A P T A T F R G H A G D D K K T K R   91
HmaS19   P F M L G H Y L G E F Q L T R S S V E H G Q A G I G A T R S S K F V P L K   140
```

Fig. 6. Comparison of the amino acid sequence with those of two eubacterial ribosomal proteins, EcoS19 from *E. coli* and BstS19 from *B stearothermophilus*, and one archaebacterial ribosomal protein HmaS19 from *H marismortui*. Identical amino acids in all four proteins are enclosed in boxes.

## 4. DISCUSSION

In this study, we first examined the number of the 30S ribosomal proteins of *M. bovis* BCG using two-dimensional gel electrophoresis. In the system used, we routinely identified 18 protein spots. Hence, the number of ribosomal proteins from the *M. bovis* BCG 30S subunits was found to be almost the same as those of typical eubacteria, such as *E. coli* and *B. stearothermophilus*.

Next, a combination of RP-HPLC and SDS-PAGE followed by a blotting procedure was employed to determine the N-terminal sequences of 13 *M. bovis* BCG ribosomal proteins. In turn, these sequences were correlated with those of the well-characterized *E. coli* and *B. stearothermophilus* ribosomal proteins.

As mentioned above, we plan to sequence *M. bovis* BCG ribosomal proteins by a combination of protein and DNA sequencing. As the initial step in this direction, following amplification of part of the MboS19 gene using two mixed synthetic oligonucleotide primers, we cloned this gene and determined its nucleotide sequence. The choice of this gene is justified by the following reasons: The protein MboS19 can be readily isolated in a pure form and with a high recovery by RP-HPLC and therefore information concerning its internal amino acid sequence can be easily obtained. The sequence of the 15 amino acids at the N-terminus of MboS19 was found to be highly conserved with respect to those of EcoS19 and BstS19 suggesting that the amino acid sequence of the peptides derived from its enzymatic digestion could be easily localized within the MboS19 primary structure on the basis of sequence homology. Since in *E. coli* [33] and in the other eubacterial [31] and archaebacterial genomes [32], the S19 gene (*rpsS*) is located within the S10 operon together with 10 other ribosomal protein genes, it could be expected that upon cloning the MboS19 gene, other *M. bovis* BCG ribosomal protein genes could rapidly be identified and sequenced. A preliminary sequence analysis of the 5′- and 3′-flanking regions of the MboS19 gene revealed the translational stop codon TAG, 6 bases upstream from the initiation codon of the MboS19 gene, and start codon ATG which overlaps with the stop codon of the MboS19 gene (TGA), respectively. The sequence analysis of the 8-kb fragment, which is presently under way in our laboratory, should provide the complete sequences of the other neighboring ribosomal protein genes.

*Acknowledgements* We thank H. Yukitake for skillful assistance and Dr. C. Gualerzi for critical reading of the manuscript This work was partly supported by a grant from the Sasagawa Memorial Health Foundation.

## REFERENCES

[1] Minden, P., Kelleher, P.J. and Freed, J.H. (1984) Infect. Immun. 46, 519–525.
[2] Minden, P., Houghten, R.A. and Spear, J.R. (1986) Infect. Immun. 53, 560–564.
[3] Closs, O., Harboe, M., Axelsen, N.H , Bunch-Christensen, K and Magnusson, M (1980) Scand. J. Immun. 12, 249–264
[4] Schou, C., Yuan, Z.L. and Anderson, A.B. (1985) Acad. Pathol. Microbiol. Immunol. Scand. Sect. C93: 265–272.
[5] Wiker, H.G., Harboe, M. and Bonnedsen, J. (1988) Scand. J. Immunol. 27, 223–239.
[6] Baker, R.E., Hill, W.E. and Larson, C L. (1972) Infect. Immun. 6, 258–265
[7] Baker, R.E., Hill, W E. and Larson, C.L. (1973) Infect. Immun. 8, 236–244.
[8] Loge, R.V., Hill, W.E., Baker, R E. and Larson, C.L (1974) Infect. Immun 9, 489–496.
[9] Ortiz-Ortiz, L., Solarolo, E.B and Bojalil, L.F. (1971) J. Immun 107, 1022–1026.
[10] Youmans, G P. and Youmans, A.S (1969) J. Bacteriol. 97, 134–139.
[11] Gregory, R.L (1986) Rev. Infect. Dis. 8, 208–217.
[12] Yamada, T (1982) FEBS Lett. 142, 267–270
[13] Suzuki, Y., Yoshinaga, K., Ono, Y., Nagata, A. and Yamada, T. (1987) J. Bacteriol. 169, 839–843.
[14] Suzuki, Y., Nagata, A., Ono, Y and Yamada, T (1988) J. Bacteriol. 170, 2886–2889.
[15] Hofman, J.D., Lau, R H. and Doolittle, W F. (1979) Nucleic Acids Res. 7, 1321–1333
[16] Ulbrich, N., Kumagai, I. and Erdmann, V.A. (1984) Nucleic Acids Res. 12, 2055–2060
[17] Sawada, M., Osawa, S., Kobayashi, H., Hori, H and Muto, A. (1981) Mol. Gen. Genet. 182, 502–504.
[18] Suzuki, Y., Mori, T., Miyata, Y. and Yamada, T. (1987) FEMS Microbiol. Lett 44, 73–76
[19] Eiglmeier, K., Honore, N., Woods, S.A , Caudron, B. and Cole, S.T (1993) Mol. Microbiol. 7, 197–206.
[20] Bercovier, H., Kafri, O and Sera, S (1986) Biochem. Biophys. Res. Commun. 136, 1136–1141.
[21] Suzuki, Y and Yamada, T. (1988) Microbiol. Immunol. 32, 1259–1262
[22] Suzuki, Y., Nagata, A and Yamada, T. (1991) Antonie van Leeuwenhoek 60, 7–11.
[23] Yamada, T., Masuda, K., Shoji, K. and Hori, M. (1972) J. Bacteriol. 112, 1–6.
[24] Hindennach, I., Kaltschmidt, E and Wittmann, H G. (1971) Eur. J. Biochem. 23, 12–16.
[25] Geyl, D., Bock, A and Isono, K. (1981) Mol Gen. Genet. 181, 309–312.
[26] Laemmli, U.K. (1970) Nature 227, 680–685.
[27] Hattori, M. and Sasaki, Y. (1986) Anal. Biochem. 152, 232–238.
[28] Altschul, S.F., Gish, W., Miller, W., Myer, E.W. and Lipman, D.J. (1990) J. Mol. Chem. 215, 403–410.
[29] Yaguchi, M. and Wittmann, H.G. (1978) FEBS Lett. 88, 227 230.
[30] Hirano, H., Eckart, K., Kimura, M. and Wittmann-Liebold, B. (1987) Eur. J Biochem. 170, 149–157.
[31] Kromer, W., Hatakeyama, T. and Kimura, M (1990) Biol. Chem. Hoppe-Seyler 371, 631–636.
[32] Arndt, E., Kromer, W. and Hatakeyama, T. (1990) J. Biol. Chem. 265, 3034–3039.
[33] Zurawiski, G. and Zurawiski, S.M. (1985) Nucleic Acids Res. 13, 4521–4526.