IDENTIFICATION AND CHARACTERIZATION OF A NEW METHYLATED AMINO ACID IN RIBOSOMAL PROTEIN L33 OF ESCHERICHIA COLI

C.N. Chang, Michael Schwartz and F. N. Chang

Department of Biology
Temple University
Philadelphia, Pennsylvania 19122

Received August 30, 1976

SUMMARY

Methylated amino acids from ribosomal protein L33 of various Escherichia coli strains (Q13, B and MRE600) were analyzed. It was found that while protein L33 from E. coli Q13 contains two methylated neutral amino acids (peaks I and II), only one methylated neutral amino acid (peak I) was found in protein L33 derived from both E. coli strains B and MRE600. The methylated amino acid present in peak I was identified as N-monomethylalanine by ion-exchange column chromatography, high-voltage paper electrophoresis and descending paper chromatography using different solvent systems. This marks the first time that N-monomethylalanine was found in any ribosomal protein.

INTRODUCTION

In our previous studies on the methylation of ribosomal proteins from [^{14}C-methyl]methionine-labeled cells, a new ^{14}C-methyl-containing peak was observed in protein L33 hydrolysate from Escherichia coli strain Q13 (1). This new peak migrated slightly toward the cathode when electrophoresis was carried out in sodium borate buffer at pH 9.3 (see Fig. 7 of reference 1). As also indicated previously in the same paper, this new peak in protein L33 separated into two unidentified methylated amino acids when protein L33 hydrolysate was analyzed in an ion-exchange column. In this communication, we would like to report that methylation patterns of protein L33 vary with different E. coli strains. While E. coli Q13 (a K strain) contains two methylated neutral amino acids, both E. coli strains B and MRE600 (which is related to a C strain) contain only one methylated amino acid. Furthermore, we have characterized this new methylated amino acid in protein L33 present in all E. coli strains to be N-monomethylalanine.

MATERIALS AND METHODS

[^{14}C-methyl]methionine (specific activity 56 mCi/mmol) was obtained from New England Nuclear and [^{14}C]alanine (specific activity 50 mCi/mmol) was a product of Schwarz/Mann. N-monomethylalanine and α-aminoisobutyric acid were purchased from Sigma Chemical Co. N,N-dimethylalanine was synthesized according to the procedure of Bowman and Stroud (2) as suggested by Bailey (3). N,N-dimethylalanine was detected by spraying the chromatogram with either thymol blue (0.04%) or orcinol (0.1%) in a mixture of n-butanol and ethanol (1:1) which was made 0.01 N with respect to H_2SO_4 (3). Biosolve BBS-3 was a product of Beckman Instruments, Inc. All reagents were of analytical grade and obtained either from Sigma Chemical Co. or local sources.

The growth and harvesting of E. coli cells in the presence of [^{14}C-methyl]-methionine were described previously (1). To obtain [^{14}C]alanine-labeled cells, E. coli Q13 was grown in 10 ml of a Tris-buffered medium (0.1 M Tris-HCl, 1 mM $MgCl_2$, 0.01 mM $FeCl_3$, 0.1 mM $CaCl_2$, 1 mM KH_2PO_4 and 0.32 mM Na_2SO_4 adjusted to a final pH of 7.4) supplemented with 0.2% glucose, 2 μg/ml thiamine, 2.0 μg/ml of each of the 20 amino acids and 5 μCi of [^{14}C]alanine. Cells were harvested at late log phase.

The preparation of 70S ribosomes, the isolation of protein L33 by two-dimensional polyacrylamide gel electrophoresis and the hydrolysis of proteins were described previously (1). Electrophoresis was carried out in 0.05 M sodium borate buffer (pH 9.3) at 2,000 volts for either 70 min (as in Fig. 3) or 180 min (as in Fig. 4). The location of amino acids and the counting of radioactivity were also described previously (1).

The analysis of methylated amino acids by ion-exchange column was run in a Beckman PA-28 resin (0.9 x 45 cm) according to the Beckman 121 manual (physiological program). The amino acids were initially eluted with 0.2 M sodium citrate buffer (pH 3.28) and changed to a 0.2 M sodium citrate buffer (pH 4.25) before the appearance of the valine peak. The basic amino acids were eluted with 0.2 M NaOH and the column was then re-equilibrated with 0.2 M sodium citrate buffer (pH 3.28). The temperature of the run was 55°. The stream-splitting procedure was used: Five-sevenths of the eluate was collected in a fraction collector and the remaining two-sevenths went into the coil to be recorded. The radioactivity (0.8 ml fractions) was detected in 10 ml of a toluene-based scintillation fluid (4 g of Omnifluor per liter of toluene) with 10% of BBS-3 (by volume).

RESULTS AND DISCUSSION

E. coli Q13 cells were labeled with [^{14}C-methyl]methionine and protein L33 was isolated by two-dimensional polyacrylamide gel electrophoresis. Fig. 1 shows the ion-exchange column analysis of protein L33 hydrolysate. It is evident that two radioactive peaks were observed. Peak I migrated between serine and glutamic acid and peak II between proline and glycine. Similar analysis of protein L33 hydrolysates from E. coli MRE600 showed that only peak I was present (Fig. 2). Peak II was not found. The methylated amino acid pattern of proteins L33 from E. coli strain B also showed only the presence of peak I (figure not shown).

Comparative studies on ribosomal proteins of four different strains of

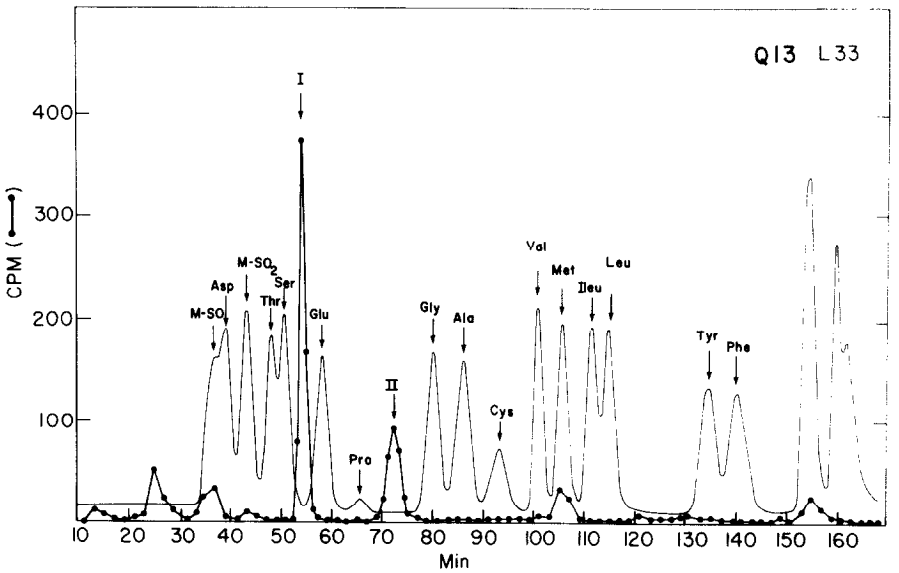

Figure 1. Ion-exchange column analysis of the methylated amino acids from protein L33 of E. coli Q13. Protein L33 hydrolysates were dissolved in 0.2 ml of 0.2 M sodium citrate buffer (pH 2.0) together with 0.06 ml of the standard amino acids (2.5 nmoles/ml, Pierce Chemical Co.), methionine sulfoxide (M-SO) and methionine sulfone ($M-SO_2$). The above mixture was then applied to a Beckman automatic amino acid analyzer (Model 121). The conditions for the ion-exchange chromatography were described in Materials and Methods. The radioactivity (0.8 ml fractions) was detected in a toluene-based scintillation fluid with 10% Biosolve BBS-3 solubilizer. The solid line represents the absorbance at 570 nm.

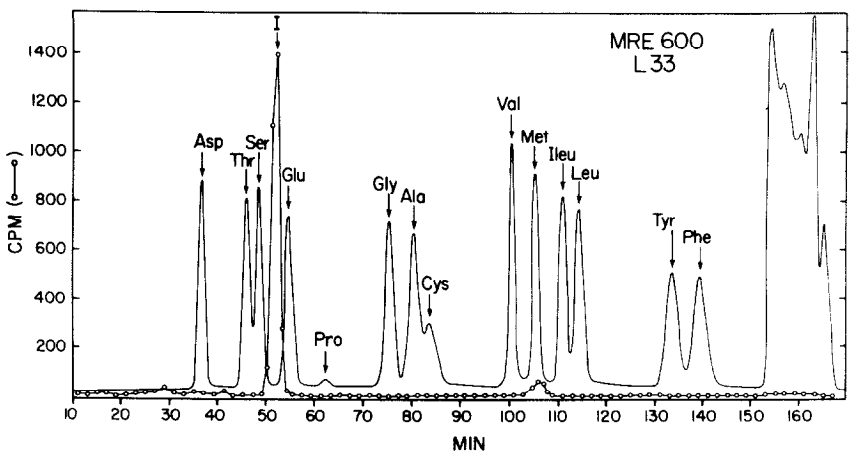

Figure 2. Ion-exchange column analysis of the methylated amino acid derived from protein L33 of E. coli MRE600. The conditions were the same as described in the legend of Fig. 1 except protein L33 hydrolysates from E. coli MRE600 were used.

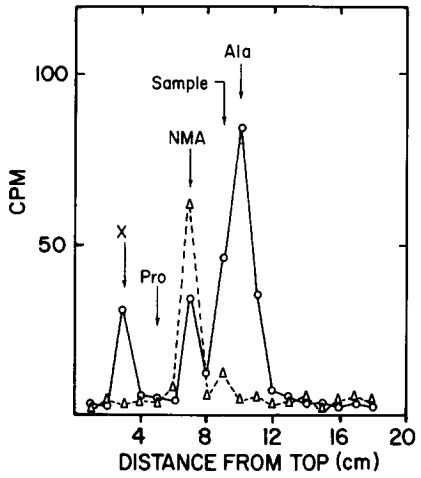

Figure 3. Analysis of the methylated amino acids derived from protein L33 of E. coli Q13 by high-voltage paper electrophoresis in 0.05 M sodium borate buffer (pH 9.3). 20 µl of the hydrolysates from [^{14}C]alanine-labeled protein L33 was applied to a Whatmann No. 3MM paper (15 x 57 cm) at 28 cm from one end together with the following amino acids: alanine, N-monomethylalanine (NMA) and proline. Electrophoresis was carried out at 2,000 V for 70 min . After Electrophoresis, the paper was dried and sprayed with 0.4% ninhydrin in acetone and followed by heating to 70° for 5 min in an oven to locate the amino acids. The paper was then cut into 1.0 cm strips and counted in a toluene-based scintillator fluid (4 g of Omnifluor/ 1 of toluene). The dashed line indicates the mobility of the purified peak I obtained from the protein L33 hydrolysate of [^{14}C-methyl]-methionine-labeled cells electrophorized side by side on the same sheet of the paper. The preparation of purified peak I was described in Materials and Methods. Sample indicates the point of application of the sample. The nature of the peak X is unknown. The cathode is to the left.

E. coli (strains B, C, K12 and MRE600) were carried out by Kaltschmidt et al (4) and found to be indistinguishable in their 50S ribosomal protein components by two-dimensional polyacrylamide gel electrophoresis. Thus the presence of peak II in E. coli Q13 and its absence in strains B and MRE600 represents the first case where a difference in a 50S protein between different strains was observed.

To determine the nature of the methylated amino acid (peak I) of protein L33 in E. coli Q13, cells were labeled with each of the following radioactive amino acids --glycine, alanine, proline, valine, isoleucine, leucine, lysine, arginine and histidine. Of all the above tested amino acids, only cells

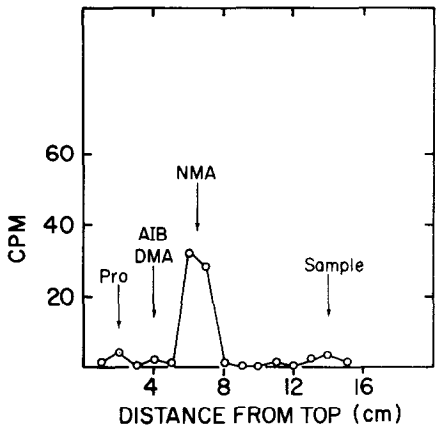

Figure 4. Identification of N-monomethylalanine from the protein L33 hydrolysate of E. coli Q13. Purified peak I was mixed with the following amino acids: N-monomethylalanine, α-aminoisobutyric acid (AIB), N,N-dimethylalanine (DMA) and proline. The mixture was electrophorized as described in the legend of Fig. 3 except the time of electrophoresis was 180 min instead of 70 min. N,N-dimethylalanine was detected as described in Materials and Methods.

labeled with [^{14}C]alanine gave rise to the methylated neutral amino acid (marked NMA) when protein L33 hydrolysates were electrophorized in sodium borate buffer pH 9.3 (Fig. 3).

Having established that peak I is a methylated derivative of alanine, we then proceeded to characterize the nature of this compound. Since methylation could occur at either the α-NH_2 group (to produce either N-monomethylalanine (NMA) or N,N-dimethylalanine (DMA)) or at the α-carbon (to generate α-aminoisobutyric acid (AIB)), these three methylated derivatives of alanine were tested. Purified peak I was obtained from a [^{14}C-methyl]methionine-labeled protein L33 hydrolysate after electrophoresis in sodium borate buffer. The regions corresponding to peak I on the paper (not sprayed with ninhydrin) were cut out, extracted with water and concentrated by evaporation in vacuo. The purified peak I was then individually mixed with each of the above three methylated derivatives of alanine and electrophorized in sodium borate buffer (pH 9.3). Of the three standards, only N-monomethylalanine co-migrated with the purified peak I (Fig. 4). Both N,N-dimethylalanine and α-aminoisobutyric

acid migrated faster than peak I (toward the cathode). It should also be pointed out that both N-monomethylglycine (sarcosine) and N,N-dimethylglycine migrated slower than peak I and can be ruled out (figure not shown). These two latter compounds were also excluded by the absence of radioactivity co-migrating with peak I in protein L33 hydrolysate labeled with [^{14}C] glycine as indicated above. A more highly methylated derivative such as N,N-dimethyl-α-aminoisobutyric acid was also ruled out since it migrated faster than proline (to the left of proline in Fig. 4).

Since this is the first time that N-monomethylalanine was found in a ribosomal protein, the following procedures were used to confirm that peak I is N-monomethylalanine. The purified peak I co-migrated with standard N-monomethylalanine in the ion-exchange column analysis identical to that reported in Fig. 1 (i.e., exactly halfway between serine and glutamic acid). The purified peak I also co-chromatographed with N-monomethylalanine in descending paper chromatography using different solvent systems: n-butanol-acetic acid-water (4:1:4, v/v) and pyridine-acetone-3 M NH_4OH (10:6:5, v/v). Thus it is concluded that peak I is N-monomethylalanine.

The molecular weight and the mole % alanine in protein L33 have been reported and using molecular weight of approximately 9,750 (5) and 4.4 mole % alanine (6), it can be calculated from Fig. 3 that the amount of N-monomethylalanine is approximately 0.8 molecules per molecule of protein L33. This number can only be regarded as tentative at present because of the uncertainty in several of the parameters used for its calculation. Since an α-N-methyl-amino acid will most likely be located at the NH_2-terminus, this indicates that protein L33 starts with N-monomethylalanine. The presence of nearly one molecule of N-monomethylalanine in protein L33 also suggests that this methylated amino acid may be required for functioning of this protein. The amount of peak II in protein L33 from E. coli Q13 is about half that of peak I, thus, there may be heterogeneity of this unknown methylated amino acid in E. coli Q13. Since peak II is present only in E. coli Q13, it is not known whether

the amount of this compound varies with the growth cycle of E. coli. The remote possibility that peak II may be due to a contaminating protein which co-migrated with protein L33 in E. coli Q13 but not in strains B or MRE600 during two-dimensional polyacrylamide gel electrophoresis has not been completely ruled out. The nature of this compound is currently under investigation.

ACKNOWLEDGEMENTS

We would like to thank Inez Navickas for her participation in part of the experiments and Carol Budzilowicz for her expert technical assistance. This investigation was supported by a U.S. Public Health Service Research Grant GM-19302.

REFERENCES

1. Chang, C. N. and Chang, F. N. (1975) Biochemistry, 14, 468-477.
2. Bowman, R.E. and Stroud, H.H. (1950) J. Chem. Soc. P. 1342-1349.
3. Bailey, J.L. (1967) Techniques in Protein Chemistry, second revised and expanded edition. Elsevier Publishing Co., p. 215-216.
4. Kaltschmidt, E., Stöffler, G., Dzionara, M., and Wittmann, H.G. (1970) Mol. Gen. Genet. 109, 303-308.
5. Dzionara, M., Kaltschmidt, E., and Wittmann, H.G. (1970) Proc. Nat. Acad. Sci., 67, 1909-1913.
6. Kaltschmidt, E., Dzionara, M., and Wittmann, H.G. (1970) Mol. Gen. Genet., 109, 292-297.