THE ENZYMATIC ACETYLATION OF E. COLI RIBOSOMAL PROTEIN L_{12}

Nathan Brot and Herbert Weissbach

Roche Institute of Molecular Biology
Nutley, New Jersey 07110

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SUMMARY

The acetylation of ribosomal protein L_{12} has been demonstrated using a soluble extract of E. coli.

INTRODUCTION

It has been previously reported that the 50S ribosomal subunit contains two copies of a protein which are identical except that one contains an N-acetyl group on the amino terminal serine (1,2). These two proteins designated L_7 (acetylated) and L_{12} (non-acetylated) by Kaltschmidt and Wittmann (3) have been shown to be required for elongation factors Tu (EF-Tu) and G (EFG) to function (4-6). The enzymatic system responsible for the acetylation has not previously been demonstrated and it is not apparent why only half of the molecules are acetylated. Acetylation could occur after the protein is incorporated into the ribosome structure but steric factors might prevent acetylation of one of the protein chains. Alternatively, both chains could be acetylated independent of the ribosome but once incorporated into the ribosome, deacetylation of one of the chains could occur.

The present report describes the enzymatic acetylation of ribosomal protein L_{12} by a soluble factor in the absence of ribosomes.

MATERIALS AND METHODS

A number of ribosomal proteins were removed from NH_4Cl-washed ribosomes (7) of E. coli using an ethanol-NH_4Cl extraction procedure previously described (4,5). A mixture of proteins L_7 and L_{12} was purified from other proteins by carboxymethylcellulose chromatography (5) and the contaminating proteins were eluted from the carboxymethylcellulose with 0.2 M sodium acetate (5) and are designated as basic proteins in Table II. L_7 and L_{12} were resolved from each other using the procedure described by Möller et al. (1).

A cell-free extract of E. coli Q_{13} was obtained by suspending 50 gms of the organism in an equal volume of a buffer containing Tris-acetate pH 7.8 10 mM, Mg acetate 14 mM, NH_4 acetate 50 mM and dithiothreitol 1 mM. The cells were disrupted in a French pressure cell and the lysate was centrifuged at 30,000 x g (S-30) for 30 minutes and then incubated and dialyzed overnight as described by Nirenberg (8). Dialysis was essential since nondialyzed samples showed little activity due to the presence of inhibitors in the extract. This dialyzed S-30 preparation was centrifuged at 100,000 x g (S-100) for two hours and the supernatant used as the source of the acetylation enzyme. ^{3}H-acetyl-coenzyme A (900 mc/mM) was purchased from the New England Nuclear Corp., and early log E. Q_{13} cells were obtained from the General Biochemical Corp.

Acetylation Reaction - Each reaction mixture contained in a total volume of 50 µl: 50 mM Tris-Cl, pH 7.4; 10 mM $MgCl_2$, 10 mM NH_4Cl, 25 mM KCl, 10 µM ^{3}H-acetyl-coenzyme A, cell-free extract, and ribosomal proteins L_7 and L_{12} as indicated in the tables and figures. Unless indicated otherwise, incubations were for 5 min at 37°. At the end of the incubation the reaction mixture was diluted with 3 ml of a cold buffer containing 10 mM each of Tris-Cl

pH 7.4, $MgCl_2$ and NH_4Cl and passed through a nitrocellulose filter (HAWP 0.45 μ, Millipore Corp.). The incubation tube was rinsed three more times with 3 ml aliquots of the buffer and then the filter was dissolved in a scintillation fluid described by Bray (9) and assayed for radioactivity in a Beckman LS-100 spectrometer. Under these conditions ribosomal proteins L_7 and L_{12} are quantitatively retained by the filter. Essentially the same results are obtained if the reactions were stopped with 10% TCA and the precipitated proteins collected on a nitrocellulose filter.

RESULTS AND DISCUSSION

Table I shows that when a mixture of ribosomal proteins L_7 and L_{12} is incubated with an S-100 preparation and ^{3}H-acetyl-coenzyme A and filtered

TABLE I

Requirements for the Acetylation of a Mixture of Ribosomal Proteins L_7 and L_{12}

Omission or Addition	^{3}H-Acetyl Protein
	pmoles
Complete	21.2
- S-100	0
- L_7, L_{12}	0.3
- S-100 + S-30	21.7
- L_7, L_{12} + 70S Ribosomes	1.0
- L_7, L_{12} + Ethanol-NH_4Cl-treated 70S Ribosomes	0.8

Details of the incubation and assay are described in the text. The complete incubations contained 584 pmoles of a mixture of L_7 and L_{12}, and either 350 μg of an S-30 or 150 μg of the S-100 preparation. Where indicated 260 pmoles of either control 70S ribosomes or ethanol-NH_4Cl-treated 70S ribosomes were added.

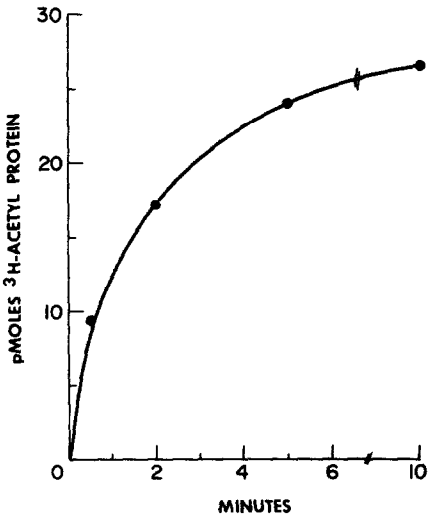

Fig. 1: Effect of time on the acetylation of ribosomal proteins L_7 and L_{12}. Details of the incubation and assay are described in the text. Each incubation contained 264 pmoles of L_{12}, and 150 μg of protein of an S-100 preparation. The incubations were carried out at 37° for the times indicated.

through a nitrocellulose filter, radioactivity is retained by the filter. This reaction is dependent on both the S-100 extract and the ribosomal proteins. Similar results were obtained when the S-30 extract was used in place of the S-100 preparation. Control ribosomes containing L_7 and L_{12} or ethanol-NH_4Cl-treated ribosomes could not replace the L_7, L_{12} mixture.

Figure 1 shows that the reaction increases with time and is essentially complete after five minutes of incubation. Since L_7 is already acetylated at the N-terminal position it would be expected that only L_{12} would be a substrate for the observed reaction. Accordingly, L_7 and L_{12} were resolved from each other (1) and assayed for their ability to be acetylated.

Table II shows that the acetylation reaction occurs only in the presence of L_{12} and that L_7 neither reacts nor has any effect on the reaction when added to an incubation containing L_{12}. Since the two proteins are identi-

TABLE II

Requirements for the Acetylation of Ribosomal Protein L_{12}

Omission or Addition	^{3}H-Acetyl Protein
	pmoles
Complete	23.9
- S-100	0
- L_{12} + L_7	0.3
- L_{12} + Basic ribosomal proteins	0.2
+ L_7	24.4

Other details of the incubation and assay are described in the text and in Table I. The complete incubation contained 264 pmoles (4.4 μg) of L_{12} protein 150 μg of an S-100 preparation, and where indicated, 5 μg of basic ribosomal proteins and 210 pmoles of L_7 were added.

cal except for the presence of an acetylated N-terminal serine on L_7, this specificity suggests that the reaction being observed is the acetylation, on L_{12}, of the N-terminal serine moiety. Although definite identification of the product has not been obtained, preliminary experiments have shown that the product of the acetylation reaction migrates with the same mobility as L_7 by disc gel electrophoresis at pH 4.5 (10). Table II also shows that other ribosomal proteins are not substrates for the enzyme.

The results presented here show that the acetylation of ribosomal protein L_{12} can occur in a ribosome-free supernatant, and that L_{12} bound to ribosomes cannot be used as substrate. Since the acetylation reaction appears to take place in the soluble fraction of the cell, it would be expected that all of the L_{12} molecules initially formed are substrates and acetylated to L_7.

One reasonable explanation for the fact that there is an equal mixture of L_7 and L_{12} on the ribosomes is that after conversion of L_{12} to L_7 in the soluble fraction, the L_7 protein binds to the ribosome and a specific deacetylation of one the L_7 chains on each ribosome occurs.

The reason for the acetylation of L_{12} is not known, but this reaction could represent a control mechanism for either protein or ribosomal RNA synthesis. Further studies are in progress to purify the enzyme(s) involved in the acetylation of ribosomal protein L_{12} and to understand how approximately equivalent amounts of L_7 and L_{12} appear on the ribosome.

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