ACETYLASE OF RIBOSOMAL PROTEIN L12: CONSTANT LEVEL OF ACTIVITY DURING THE GROWTH CYCLE

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1. Introduction

Many proteins which in living cells perform diverse functions, e.g., vertebrate cytochrome *c* [1], muscle actin [2], several histones [3], are known to have their terminal α-amino groups modified by acetylation. In *Escherichia coli*, a protein in the large subunit of ribosome exists in both α-*N*-acetylated (L7) and unmodified (L12) forms [4,5]. This protein is involved in all the factor-dependent GTP hydrolysis steps carried out by the ribosome during protein biosynthesis (reviewed in [6]).

The specific function of acetyl modification in the ribosome system (as also in the other systems) remains yet unknown [7]. However, in *E. coli* it has been found that the amount of L7 and L12 in ribosomes depends on growth rate [4,8] and that their ratio undergoes a large, unimodal variation during the growth cycle in rich medium with no apparent change in the sum of L7 + L12 per ribosome [9]. More recently it has been further shown that both L7 and L12 are utilized for ribosome-assembly throughout the growth cycle and that the above variation takes place by changes in the relative flow of L7 and L12 species into ribosome-assembly rather than by modification in pre-existing ribosomes [10].

A possible mechanism for controlling the relative flow of L7 and L12 is through changes in the activity of L12-acetylase, the enzyme which transfers acetyl group to L12 from acetylCoA [11]. If such were the mechanism of control, the activity of the enzyme in the cell would parallel the level of L7 in ribosomes and would increase as a culture approaches stationary phase. We have determined the level of L12-acetylase in extracts from *E. coli* cells harvested at different points in the growth cycle. We find that the level *remains constant*.

2. Materials and methods

E. coli (strain MRE 600) was grown with aeration at 37°C in L-broth, harvested, washed and frozen at −80°C as previously described [9,12]. The cells were broken by grinding with alumina, extracted with TKM buffer (0.01 M Tris–HCl, pH 7.8, 0.05 M KCl, 0.01 M $MgAc_2$, 0.007 M 2-mercaptoethanol, 4 ml/g cell) in the presence of 0.5 μg/ml pure DNase (Worthington Biochemicals) and ribosomes were pelleted by centrifugation at 45 000 rev/min for 4 hr (Beckman 50 Ti rotor). The supernatant (excluding about 0.5 ml at the bottom) after dialysis was used for L12-acetylase assay. The ribosomal pellet was dissolved in a small volume of TKM buffer, proteins extracted with acetic acid [13], and L7/L12 ratio determined by polyacrylamide gel electrophoresis and densitometry as described [14].

L12-acetylase was assayed by the procedure of Brot and Weissbach [11] with modifications indicated in Results. The incubation mixture (50 μl) contained 1.4 μg L12 (2.4 μM), 0.5 μCi of [^{3}H] acetylCoA (14 μM), buffer and 2-mercaptoethanol (see Results). After incubation, the reaction mixture was diluted, filtered through Millipore and the retained [^{3}H] acetyl-L12 was counted with Brays mixture in a scintillation counter.

Brot et al. [16] have shown that [^{3}H] acetyl-L12 produced in vitro co-electrophorese with L7. We have also found that 82% of the counts co-electrophorese with L7 in the present system [4].

The L12 preparation was made from mid-log phase ribosomes by ethanol–NH_4Cl extraction [16]. It contained 28% L7, but L7 has no apparent effect in the assay [11]. Protein concentrations were determined by the procedure of Lowry et al. [17] with crystalline bovine serum albumin standard. [^{3}H] acetylCoA (740 Ci/mol) was purchased from New England Nuclear and cyclic AMP from Sigma.

3. Results

3.1. *Assay of L12-acetylase:heat lability*

Brot et al. [15] have noted that L12-acetylase in cell extracts is unusually heat labile and that incubation in the absence of substrates at 37°C (their assay temperature) leads to rapid loss in activity. We, therefore, used 30°C for our assay. At this temperature the reaction (with 40 μg or less of supernatant protein in assay mixture) was linear for about 2 hr. The enzyme was unstable even at this temperature. Preincubation of the enzyme extract at 30°C led to about 25% loss in 30 min and 70% loss in 2 hr (table 1). Complete protection was provided by acetylCoA, but interestingly, the addition of L12 which is also a substrate afforded only slight protection (table 1).

The preceding assays were performed in 0.01 M Tris–HCl, pH 7.8, buffer containing 0.002 M 2-mercaptoethanol (see below) since no specific requirement for Mg^{++} or K^+ was found. Indeed, high concentrations of these ions (or $Tris^+$) were progressively inhibitory as already noted [15].

Table 1
Heat-lability of L12-acetylase

Duration of exposure to 30°C	Addition prior to exposure	L12-acetylase activity remaining	
		[^{3}H]acetyl cpm	Percent activity
0 min	–	4301	100
30 min	–	3398	76
60 min	–	2842	61
120 min	–	1679	30
120 min	Acetyl CoA	4125	95
120 min	L12	1964	37
	Blank = no enzyme	567	0

15 μg of supernatant proteins (in 45 μl of 0.01 M Tris–HCl, pH 7.8, 2 mM 2-mercaptoethanol), with or without 14 μM acetyl CoA or 2.4 μM L12 (same concentration as in the assay) was exposed to 30°C and immediately assayed for L12-acetylase activity.

Table 2
Requirement of -SH protection by L12-acetylase

Treatment or addition	L12-acetylase activity remaining	
	[^{3}H]acetyl cpm	Percent activity
A. None	4193	100
Dialysis, 0.01 M Tris, no ME	1048	14[a]
Dialysis, 0.01 M Tris, no ME	1584	29[b]
Dialysis, 0.01 M Tris, 2 mM ME	4367	105
Blank = no enzyme	516	0
B. No addition	5089	100
2×10^{-3} M PCMB	473	1
2×10^{-3} M NEM	2719	51
Blank = no enzyme	430	0

Tris = Tris–HCl, pH 7.8; ME = 2-mercaptoethanol; PCMB = *p*-chloromercury benzoate; NEM = N-ethyl maleimide. 15 μg supernatant proteins present in each assay. Experiments A and B were done with different cell supernatants. A lower concentration of 2-mercaptoethanol (1.4×10^{-3} M) was used in experiment B for obvious reasons.
[a]Assayed in the absence
[b]Assayed in the presence of 2 mM 2-mercaptoethanol.

3.2. *Requirement for -SH protection*

L12-acetylase requires protection by sulfhydryl reagents. A supernatant preparation when dialyzed against Tris buffer and assayed in the absence of 2-mercaptoethanol retained only a small fraction of its original activity (table 2). This requirement is not noticed with undialyzed preparations because the buffer used for cell extraction provides sufficient 2-mercaptoethanol in the assay. The importance of -SH groups is further shown by the abolition of enzyme activity by sulfhydryl reagents (table 2). The removal of -SH protection from L12 acetylase leads to irreversible inactivation: only a part of the lost activity was restored when the enzyme was assayed with added 2-mercaptoethanol (table 2). The activity was the same when assay mixture contained between 0.7 and 2.8 mM 2-mercaptoethanol and therefore we routinely used 2 mM concentration.

3.3. *pH-optimum for assay*

L12-acetylase was assayed in the foregoing experiments at pH 7.8 in 0.01 M Tris–HCl buffer. Experiments at other pH values showed that the enzyme possesses a rather flat pH-optimum between 6.7 and 7.5. The activity drops to 55% at pH 8.0 and to 60% at pH 6.5 (0.01 M PO_4). The assay pH of 7.4 used by other workers [11,15] was therefore satisfactory, but we chose to use pH 7.1, the mid-point of the optimal pH-range. Since Tris–HCl is a poor buffer at this pH 0.01 M PO_4 (K^+) buffer was used.

The activity level of the enzyme, expressed as picomol acetyl transferred per mg protein, in the post-ribosomal supernatant was 600–800; these values are about 100 times the activity levels reported for histone acetylase in calf thymus nuclei preparations [18]. There was neglible enzyme activity (3%) in a 1 M NH_4Cl-extract of the ribosomal pellet.

3.4. *L12-acetylase level of cells harvested at different phases of the growth cycle*

E. coli was grown in rich medium and cells were harvested at different points in the growth cycle, from early logarithmic to stationary phase (between 105 min and 6 hr after inoculation). Post-ribosomal supernatants prepared from these cells (under identical conditions) were dialyzed in the cold against 0.01 M phosphate, pH 7.1, 2 mM 2-mercaptoethanol and L12-acetylase was assayed. Ribosomal pellets from the same extracts were used for L7/L12 ratio determination. The data from all experiments are summarized in fig.1. There was, as expected, a 7-fold increase in L7/L12 ratio in ribosomes between the early-log and stationary phase cells. However, the activity level of L12-acetylase showed no increase but remained virtually constant throughout the growth cycle examined. An apparent slight decrease in the 6 hr culture, seen in fig.1, probably reflects shift in cell metabolism in non-growing cells, e.g., the supernatants from stationary phase cells showed a higher protein concentration than the supernatants from mid-log phase cells.

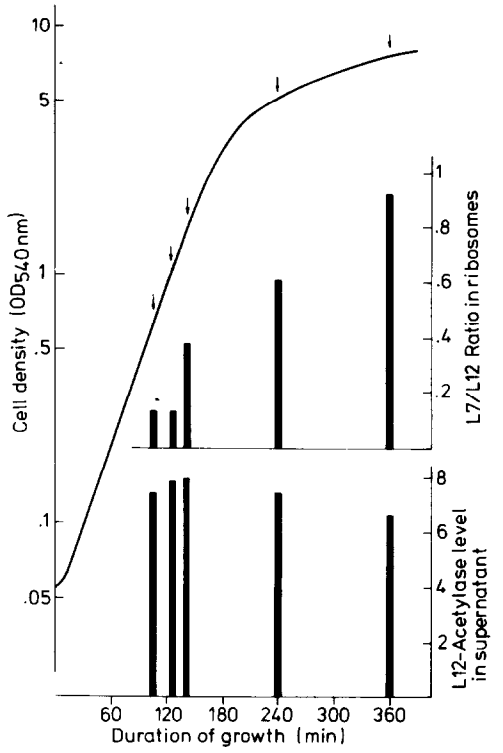

Fig.1. L12-acetylase levels and ribosomal L7/L12 ratios in *E. coli* at different parts of the growth cycle. The enzyme level expressed as pmoles L12 acetylated per mg cells (1 pmol = 602 cpm). Arrows show when cultures were harvested.

4. Discussion

Active ribosomes from *E. coli* contain both acetylated and unmodified forms of the same protein in relative amounts which depend on growth rate and growth cycle of cell. Therefore, this system raises questions, e.g., how does the cell maintain a pool of unmodified L12 in the presence of an active acetylase, how is the relative utilization of L7 and L12 regulated, which are accessible to experimental study. In this report we show that the characteristic increase in L7 content in ribosomes observed in late logarithmic and stationary phase cells is *not* accompanied by a parallel increase in the activity of L12-acetylase in cell extracts (fig.1).

It is possible that enzyme activities measured in cell extracts do not reflect their levels in vivo. The latter could be modulated by effectors which are either dialyzed out or diluted (the cytoplasm would be about 100-fold diluted in our assay mixture) in the extract. Although the accumulation of a compound in late logarithmic phase which enhances the enzyme's

activity could explain the increased production of L7, such an effector has not been found so far. Cyclic AMP is known to markedly accumulate in stationary phase [19]. However, no significant effect was noticed when L12-acetylase was assayed in the presence of (up to 10^{-3} M) cyclic AMP.

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