AMINOACYL-tRNA SYNTHETASE FORMATION AND CELL DIVISION IN BACTERIA

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1. Introduction

I have earlier proposed that the formation of aminoacyl-tRNA synthetases (EC 6.1.1) is coupled with the cell division in the cultures of *Streptococcus thermophilus* KQ. Now I have tested this hypothesis by using an electronic particle counter. The results presented in this paper show that the activities of aminoacyl-tRNA synthetases increase only in phases of a batch culture in which the total number of cells is rising.

2. Materials and methods

S. thermophilus strain KQ was grown without aeration in ST medium. The details of the culture conditions have been described [1, 2]. *Escherichia coli* U5/41 was grown in glucose-salt medium [3], which was aerated by shaking. The details have been published earlier [4].

The activities of aminoacyl-tRNA synthetases were determined by measuring the rate of the amino acid dependent ATP-PP_i exchange reaction [1, 2, 4]. Inorganic pyrophosphatase (EC 3.6.1.1) was assayed by measuring the liberation of labelled phosphate from ^{32}P-PP_i [5]. The activity of β-galactosidase (EC 3.2.1.23) was measured by using *o*-nitrophenyl-β-D-galactopyranoside as substrate [6]. Ultrasonically treated cell suspensions were used as the enzyme preparations [1, 2, 4]. The initial reaction rates versus the amounts of the enzymes gave straight lines in our assay conditions.

The total number of the bacterial cells in the cultures was measured with an electronic particle counter (Celloscope 302, AB Lars Ljungberg & Co., Stockholm, Sweden) by using an aperture of 30 μm in diameter. Because the cells of *S. thermophilus* KQ form chains, the samples were first incubated in 35% formaldehyde solution to harden the cell walls. The chains were then broken down to single cells by 30 sec ultrasonic pulse (Ultrasonic Disintegrator, 60 W 20 KHz, Medical and Scientific Equipments Ltd., London, England). The treated cell suspensions were diluted for particle counting with dust-free 0.14 M sodium chloride solution to the total count of about 10^5 particles/ml. This method, developed for this work, will be described in detail elsewhere [7]. The total number of *E.coli* U5/41 cells was counted by the identical procedure, except that ultrasonic treatment was omitted.

3. Results and discussion

In the batch culture of *S. thermophilus* KQ, the total cell number and the total activity of valyl-tRNA synthetase increase in a similar way and both reach maxima after 2 hr of growth (fig. 1A), although the cell mass does not stop increasing until after 4 hr. In the culture of *E.coli* U5/41, both the total number of cells and valyl-tRNA synthetase activity rise almost to the end of growth (fig. 2). In fig. 3, the lag and acceleration phases of the culture are illustrated in an extended scale. The total activity of the enzyme closely follows the total cell count, not the turbidity of the culture.

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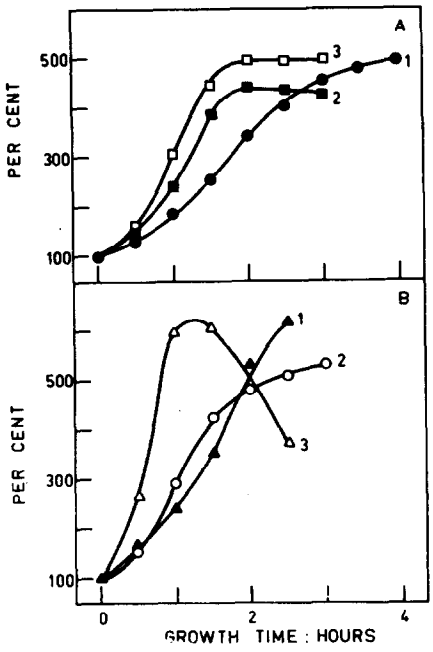

Fig. 1. Changes of the total cell number and the total enzyme activities in a batch culture of *S. thermophilus* KQ. All variables are presented as percentages of the initial values.
(A) 1. Turbidity of the culture (Klett colorimeter, filter 62);
2. total number of cells;
3. total activity of valyl-tRNA synthetase.
(B) 1. Number of large cells (volume roughly 2-fold compared with the smallest counted particle);
2. total activity of β-galactosidase;
3. total activity of inorganic pyrophosphatase.

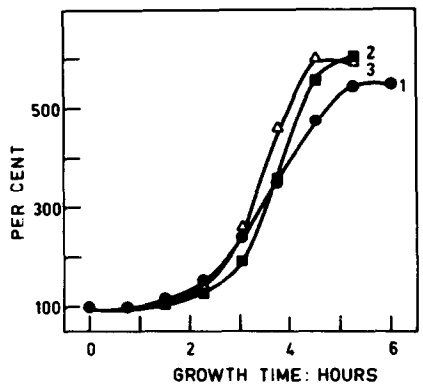

Fig. 2. Changes in the turbidity of the culture (1), in the total number of cells (2) and in the total activity of valyl-tRNA synthetase (3) in a batch culture of *E.coli* U5/41. All variables are presented as percentages of the initial values.

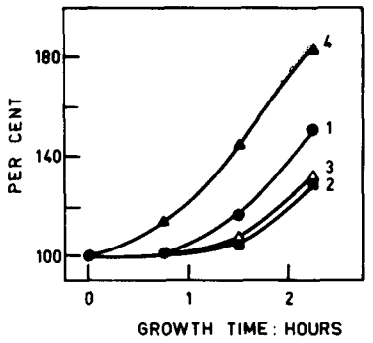

Fig. 3. The initial parts of the curves in fig. 2 drawn in an extended scale. The numbering of curves as in fig. 2. Curve 4: number of large cells.

Because *E.coli* U5/41 does not form chains, the samples were not treated by ultrasound, but were shaken vigorously to destroy the loose cell aggregates. Therefore for the good agreement between the results obtained by using *E.coli* and *S.thermophilus* KQ also show the validity of the ultrasonic treatment.

The above results suggest that the formation of valyl-tRNA synthetase activity is in some way coupled with the cell division. We found previously that the activity curves of all aminoacyl-tRNA synthetases are very similar in all growth conditions tested [1, 2, 4] Therefore it is evident that the activities of all these enzymes change in the close correlation with the cell number.

To test if the similar correlation exists for other enzymes, inorganic pyrophosphatase and β-galactosidase in the batch culture of *S.thermophilus* KQ were also assayed. These enzymes were chosen, because inorganic pyrophosphatase is known to be a constitutive enzyme, at least in *E.coli* [8], and β-galactosidase of *S.thermophilus* KQ is independent of lactose in the medium [9]. The activity curves of these enzymes did not follow the total cell count (fig. 1B).

Table 1 shows that in the constant growth conditions the enzyme activity and the total number of cells rise in the same proportion, but in the enriched cultures the activity rises more than the cell count. The activity of valyl-tRNA synthetase per cell increases in the richer media in the same proportion as the specific activity of the enzyme. This suggests that the mass of one cell does not change because of the enrichments.

Table 1
Changes in cell number and valyl-tRNA synthetase activity in the cultures of *S.thermophilus* KQ in 3 different media.

Medium	A	N	100 (A/N)	SA
ST	194	188	102	100
T	497	445	112	117
C	601	388	155	150

Cells grown in ST medium were suspended in prewarmed ST medium. The culture was immediately divided into 3 portions: (T) was enriched with 1 g of peptide mixture (Bacto tryptone, Difco) per l; (C) 1 g of amino acid mixture (Bacto casamino acids, Difco) + 5×10^{-4} moles of L-tryptophan per l were added; (ST) no additions. The maximal values of the total activity of valyl-tRNA synthetase (A) and the total cell number (N) are presented as percentages of the initial values. The maximal specific activity of the enzyme in the ST medium is arbitrarily defined as 100 and that in the other media is presented in the same units.

The mechanism that restricts the formation of aminoacyl-tRNA synthetase activity to phases of growth in which the cells are dividing is still unknown. The synthesis of protein is not under similar restriction. The turbidity of the culture increases after the cell division is completed in the batch culture of *S. thermophilus* KQ (fig. 1A), and begins to rise earlier than the number of cells in the case of *E.coli* U5/41 (fig. 3). Curve 1 in fig. 1B and curve 4 in fig. 3 show that this is due to the growth of cell volume. Also the rise in the total activity of β-galactosidase during the phase of the constant cell number (fig. 1) suggests that protein synthesis in bacterial cultures is not arrested when the total number of the cells reaches the maximum. Therefore the synthesis of aminoacyl-tRNA synthetases must be somehow prevented in the non-dividing cells. Another possibility is the balanced production and destruction of the enzymes, as observed by Williams and Neidhardt [10] in bacterial cultures as a result of the shortage of a necessary amino acid.

It will be interesting to test if close correlation between activity and cell number also occurs with other enzymes. The activity curves of β-galactosidase and inorganic pyrophosphatase (fig. 1B) show that this rule is not generally applicable even for constitutive enzymes.

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