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Formation of Aminoacyl-sRNA Synthetases during Growth Phases of *Escherichia coli*

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The activities of aminoacyl-sRNA synthetases in ultrasonically treated *Escherichia coli* cells (strain U5/41) were determined by measuring the rate of ATP-PP exchange in the presence of the respective amino acids. All the 20 amino acids tested except arginine increased the rate of ATP-PP exchange from the endogenous level. The most active were leucyl-, valyl-, isoleucyl-, tyrosyl-, tryptophanyl-, methionyl-, and phenylalanyl-sRNA synthetases in decreasing order of activity.

The activities of the mentioned aminoacyl-sRNA synthetases were assayed at various growth phases of *E. coli* U5/41. The specific activities remained constant during the lag phase, began to rise in the acceleration phase, reached maximal values in the middle of the exponential phase, and decreased to the original levels at the beginning of the stationary phase. The maximal specific activities were 1.5–2 times the initial values.

Aminoacyl-sRNA synthetases [amino acid-RNA ligases (AMP), E. C. subgroup 6.1.1.] catalyze the formation of aminoacyl-sRNA, the first step in the biosynthesis of proteins. Previously a paper [1] concerning the formation of aminoacyl-sRNA synthetases during various growth phases of *Streptococcus thermophilus* (strain KQ) has been published from this laboratory by Heinonen. In this paper we present for comparison the results of similar studies where *Escherichia coli* (strain U5/41) was used as test organism. The changes in the activity of valyl-sRNA synthetase during batch cultivation of *E. coli* B have been presented earlier by Shortman and Lehman.[2]

EXPERIMENTAL

Cultivation of the test organism. Escherichia coli (strain U5/41) was used as test organism in the experiments. Its origin and maintenance have been described earlier.[3] At the beginning of an experiment, the strain was transferred with a platinum wire from an agar slant to 5 ml of GSHT medium, which was then incubated at 37°. After 4 h, the whole medium was poured aseptically into 500 ml of GSHT medium, which was incubated 16 h at 37°. The

cells were then separated by centrifugation (3000 *g*, 10 min), washed once with cold (+4°) 0.14 M sodium chloride solution, and suspended in 0.14 M sodium chloride for storage before cultivation. The time between precultivation and an actual experiment was in most cases about 4 h.

In the actual experiments the cells were separated by centrifugation (3000 *g*, 10 min) and suspended in 1 liter of minimal medium at 37°. Cultivation took place in an open vessel with mechanical stirring. The temperature of the culture was kept at 37° by immersing the cultivation vessel in a thermostated water bath. Growth was recorded by measuring the turbidity of samples, taken at intervals from the growth medium, in a Klett-Summerson colorimeter using filter 62 (590–660 nm). The compositions of the GSHT and minimal media have been described earlier.[4]

Treatment of samples. The volume of a sample was calculated by dividing 600 by the turbidity of the growth medium. Thus the samples contained roughly equal amounts of cell mass (about 3 mg dry weight). The samples were immediately cooled in an ice-water bath and centrifuged (3000 *g*, 10 min) in 50 ml centrifuge tubes. They were then transferred to 10 ml glass tubes with about 7 ml of cold sodium chloride solution. The cells were again separated by centrifugation and the supernatant was discarded. The mouths of the tubes were dried with soft tissue paper and the tubes were stored in ethanol at −35°.

To break the cells the sample was transferred onto the oscillating membrane of an ultrasonic disintegrator (Raytheon DF 101, 250 W, 10 kc) with 15 ml of buffer solution (0.25 M Tris-hydrochloric acid buffer, pH 7.5, containing 750 mg of potassium chloride, 400 mg of magnesium chloride hexahydrate, and 200 mg of reduced glutathione per 100 ml). The disintegrator was allowed to run with constant output (0.85 A) for 4 min while cooling water was vigorously circulated to keep the temperature of the sample under 5°. The broken cells were added to a series of small test tubes, which were stored in ethanol at −35°.

Aminoacyl-sRNA synthetase assays and *protein estimations* were performed as described earlier.[1] The measured activity of each enzyme was directly proportional to the reaction time and to the amount of enzyme preparation in the conditions used in this work.

Dry weight was determined by isolating cells from a 50 ml sample by centrifugation (3000 *g*, 10 min) and washing them twice with cold 0.14 M sodium chloride solution and once with cold distilled water in tared test tubes. The tubes were dried for 8 h at 115°, cooled in a desiccator and weighed.

RESULTS AND DISCUSSION

The activities of aminoacyl-sRNA synthetases in E. coli U5/41. Table 1 shows that all twenty amino acids tested, except L-arginine, increased the rate of ATP-PP exchange over the endogenous level (no amino acid added). The activity level, however, varied greatly from one enzyme to another. Nisman [5] determined the activities of aminoacyl-sRNA synthetases in ultrasonically disrupted *E. coli* K12 cells. Also his results are presented in Table 1 for comparison. The results of both studies are in good agreement except in the case of lysyl-sRNA synthetase where they differ considerably. This may be due to the different pH values of the reaction mixtures (8.0 in the work of Nisman, 7.5 in this work) and/or to the different strains of *E. coli*.

We have presented earlier [1] the results of similar studies concerning *Streptococcus thermophilus*. DeMoss and Novelli [6] determined the activities of aminoacyl-sRNA synthetases in *E. coli*, *Streptococcus hemolyticus*, and *Neurospora crassa*. In every case leucyl-, isoleucyl-, valyl-, tyrosyl-, methionyl-, and phenylalanyl-sRNA synthetases were found to be most active. Only Hahn and Brown [7] got somewhat different results when they studied ultrasonically disrupted *Sarcina lutea* cells. Also in their study valyl-, leucyl-,

Table 1. Specific activities of 20 aminoacyl-sRNA synthetases in samples taken during the exponential phase of growth of *E. coli* U5/41 and *E. coli* K12.[5] The results of Nisman [5] have been recalculated in the same units (mμmole PP into ATP in one minute per mg of protein).

Added amino acid	Specific activity U5/41	Specific activity K12	Added amino acid	Specific activity U5/41	Specific activity K12
No aa	4.2	3.3	L-asp	3.0	0.3
L-val	43	60	L-asn	10	—
L-gly	2.5	0.2	L-met	21	19
L-glu	9.6	—	L-his	7.4	14
L-gln	2.6	—	L-phe	13	8.8
L-ser	4.6	3.5	L-try	28	40
L-thr	6.6	6.4	L-tyr	30	26
L-leu	59	61	L-pro	7.4	3.2
L-ile	37	31	L-lys	10	28
L-arg	0	3.6	L-ala	8.3	5.6
			L-cySH	8.4	10

methionyl-, and isoleucyl-sRNA synthetases were among the most active enzymes, but the activity of phenylalanyl-sRNA synthetase was low, while also cysteinyl-, threonyl-, alanyl-, histidyl-, and aspartyl-sRNA synthetases belonged to the group with highest activity. We are not able to explain why almost in every case the same aminoacyl-sRNA synthetases show the highest activity, although the respective amino acids are not most common in microbial proteins.[8] It must be remembered, however, that the relative activities of different aminoacyl-sRNA synthetases need not be equal *in vivo* and *in vitro*. Reasons for this have been discussed in detail by Heinonen in a previous paper from this laboratory.[1]

Changes in the activities of aminoacyl-sRNA synthetases during batch cultivation of E. coli U5/41. Fig. 1 presents the activities of leucyl-, valyl-, isoleucyl-, and phenylalanyl-sRNA synthetases and the growth of the bacterial mass as functions of growth time. All activity curves are very similar in form. When the cultivation time from the beginning of growth to the beginning of the stationary phase is divided into 100 equal parts,[9] the highest specific activity occurs between 40 and 50 on this scale. In the last half of the cultivation period, the specific activity decreases almost to the initial level. The rise of specific activity from the beginning to the maximum is about 1.5-fold. The activities of tyrosyl-, tryptophanyl-, and methionyl-sRNA synthetases varied similarly, but they are not plotted in Fig. 1 for the sake of clarity. Other aminoacyl-sRNA synthetases were not studied because their activities were not high enough for precise measurement.

Enzymes whose activities rise already in the lag phase of growth have been named lag-phase enzymes by Soini and Nurmikko.[9] Fig. 2, where the specific activities of isoleucyl-, phenylalanyl-, and methionyl-sRNA synthetases during the lag and acceleration phases are plotted, shows that these aminoacyl-sRNA synthetases do not belong to the group, since their activities remain constant throughout the lag phase. The activities of leucyl-, valyl-, tryptophanyl-, and tyrosyl-sRNA synthetases varied similarly.

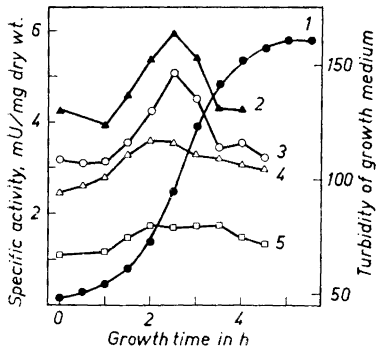

Fig. 1. Specific activities (mU/mg dry weight $\times 10^{-1}$) of some aminoacyl-sRNA synthetases during batch cultivation of *E. coli* U5/41.

Curve 1: turbidity of the growth medium (Klett-Summerson colorimeter, filter 62). Curve 2: leucyl-sRNA synthetase. Curve 3: valyl-sRNA synthetase. Curve 4: isoleucyl-sRNA synthetase. Curve 5: phenylalanyl-sRNA synthetase.

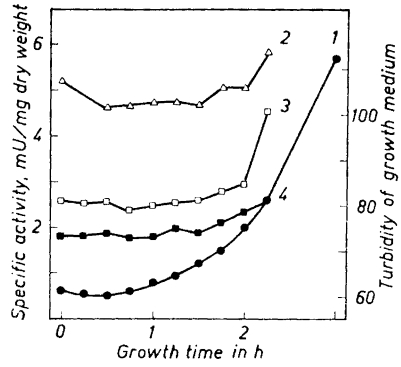

Fig. 2. Specific activities (mU/mg dry weight $\times 10^{-1}$) of some aminoacyl-sRNA synthetases during the lag and acceleration phases of *E. coli* U5/41.

Curve 1: turbidity of the growth medium (Klett colorimeter, filter 62). Curve 2: isoleucyl-sRNA synthetase. Curve 3: phenylalanyl-sRNA synthetase. Curve 4: methionyl-sRNA synthetase.

Shortman and Lehman [2] assayed the activity of valyl-sRNA synthetase during various growth phases of *E. coli* B. The stage of growth when the specific activity was maximal was in the time range 40—50, in agreement with our results. The period when the activity is maximal is somewhat longer in the activity curve presented by Shortman and Lehman. This may be due to different strains and growth media. They found the maximal specific activity to be about three times the initial activity, while in our experiments the rise was only 1.5 to 2-fold. This evidently depends on the physiological age of the cell mass used for inoculation, for we found that the rise in specific activity was the greater the longer the period of precultivation.

Hahn and Brown,[7] Pang Ting Chao and Zaitseva,[10] and Heinonen [1] determined the activities of several aminoacyl-sRNA synthetases during batch cultivation of *Sarcina lutea*, *Azotobacter vinelandii*, and *Streptococcus thermophilus*, respectively. In every case the activity was maximal in the exponential phase, at about 35 by Hahn and Brown and at 30 by Heinonen. Pang Ting Chao and Zaitseva assayed the activities at only three points during growth. Therefore we could not calculate the position of the maximal activity in this case. The absence of the lag phase of growth in the experiments of Hahn and Brown and in those of Heinonen explains why they found the specific activity to be maximal earlier than we did. The positions of the maxima are very nearly the same (30—35) if they are calculated relative to the exponential phase only.

In our experiments and in those of Hahn and Brown [7] and Heinonen [1] mentioned above, the activities of all aminoacyl-sRNA synthetase were found

to vary in a similar way. It seems thus that the formation of all aminoacyl-sRNA synthetases is coordinated in some way. It has been shown earlier by Heinonen[1] that the formation of all the enzymes is most rapid, when the total amount of free amino acids in the cells is maximal. This suggests that the size of the amino acid pool may determine the rate of synthesis of aminoacyl-sRNA synthetases. This has been proposed by Henshall and Goodwin,[11] but has been disputed by Anderson and Rowan.[12] Nass and Neidhardt[13] have shown that in certain cases a shortage of an amino acid derepresses the formation of the specific aminoacyl-sRNA synthetase. This makes it highly improbable that free amino acids directly induce the synthesis of these enzymes, and Mariani *et al.*[14] even proposed that free amino acids repress the synthesis. This proposal is in agreement with the results of Nass and Neidhardt, but disagrees with those of Heinonen. Further studies are needed to solve this problem. Such are now in progress in this laboratory.

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