METABOLIC POOL OF FREE NUCLEOTIDES OF THE BACTERIAL CELL

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The pool of free nucleotides of bacteria is known to include several tens of nucleotides and their derivatives. Some of these are precursors of nucleic acids, while other play the part of coenzymes in the biosynthesis of proteins, carbohydrates, and lipids. The importance of a third group, such as nucleotide peptides [4, 6, 7, 12], nucleoside peptides [13], adenosine-succinic acid [3], and other complex compounds has not yet been finally settled. Some assistance in determining the biological role of individual components of this pool may be given by the study of free nucleotides during exposure of the bacterial cell to certain agents (irradiation, administration of antimetabolites or antibiotics, the effect of the temperature factor, and so on). For this reason the problem of the constancy or variability of the pool of free nucleotides during changes in the conditions of cultivation and the method of obtaining the bacterial mass is thus very important.

In an earlier paper [2], the acid-soluble nucleotides were analyzed in cells of Escherichia coli strain No. 19 grown on a solid nutrient medium (meatpeptone agar). When the conditions of cultivation were changed, certain changes were observed in the picture of the free nucleotide pool.

The present paper describes the result of a study of the acid-soluble fraction of two strains of E. coli when grown in liquid nutrient media of different composition.

EXPERIMENTAL METHOD

An 18-hour culture of E. coli (strain No. 19 or 613) was seeded in flasks of meat-peptone broth or synthetic medium M9 (pH 7.3), containing per liter: 1 g NH_4Cl, 0.13 g $MgSO_4$, 3 g KH_2PO_4, and 6 g $Na_2HPO_4 \cdot 12H_2O$. Glucose was used as the source of carbon in a final concentration of 4 g/liter. The culture was incubated at 37° with aeration. At certain intervals of time samples were taken for measurements of the turbidity from the optical density at 500 mμ, and also for determination of the degree of excretion of nucleotide material into the culture fluid from the absorption of uv light by the supernatant fluid after sedimentation of the bacterial cells by centrifugation. At the end of cultivation the cells were collected by separation, washed with 0.5% NaCl solution, and extracted with 0.5M $HClO_4$ solution. All the operations from the end of cultivation were carried out in the cold. After neutralization and removal of the $HClO_4$, the $HClO_4$ extract was applied in an amount equivalent to 100 SU* to a column with Dowex 1 × 4, 200-400 mesh, in the formate form (diameter of column 0.6 cm, height 7 cm). The volume of the fractions obtained was 2.6 cm and the rate of flow through the column was 12.5 ml/8. Parr's gradient method of elution was used [9]. The extinction of the fractions was measured on the SF-4 spectrophotometer at 260 and 280 mμ, and also recorded automatically by means of the Uvicord LKB uv-absorptiometer. The fractions were free from eluent by evaporation in a film evaporator or by treatment with charcoal. The multicomponent fractions were finally separated by electrophoresis and chromatography on paper in several systems of solvent. The isolated compounds were analyzed and identified by methods described previously [2].

*SU—spectrometric units (extinction at 260 mμ in a cell with an optical section of 1 cm, multiplied by the dilution and the volume in ml); US—substances absorbing in the ultraviolet region of the spectrum; MPB—meat-peptone broth; M9—synthetic medium M9.

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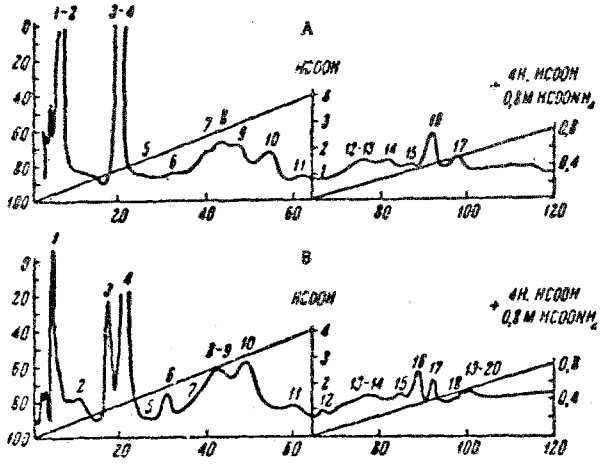

Fig. 1. Separation of free nucleotides from cells of E.coli strain No. 19, cultivated in mean-peptone broth, on a column. Here and in Fig. 2: A—stationary phase of growth; B—logarithmic phase of growth. Cclumn 0.6 × 7 cm, Dowex 1 × 4, 200-400 mesh, formate form. Gradient elution; first system: mixer—100 ml H_2O, reservoir—100 ml 4 N HCOOH solution; second system: mixture—100 ml 4 N HCOOH solution, reservoir—100 ml 0.8 M $HCOONH_4$ solution + 4 N HCOOH solution. Rate of flow 12.5 ml/h, volume of fraction 2.6 ml. Along the axis of abscissas—fractions; along the axis of ordinates—transmission (in %, 254 mμ).

EXPERIMENTAL RESULTS

The ion-exchange chromatograms of the acid-soluble nucleotides from the E. coli (strain No. 19) cells cultivated in meat-peptone broth are shown in Fig. 1. Analysis of the fractions obtained by elution from the column showed that the peaks usually contained 2, 3, or more compounds. Identification of the individual fractions by paper chromatography, electrophoresis, and other methods revealed the following details of the composition of the free nucleotides previously observed during cultivation in meat peptone agar: adenine derivatives were predominant, next followed uracil derivatives; very small amounts of guanine and, in particular, cytosine compounds were found. More monophosphates were present than diphosphates, and triphosphates were present in very small amounts.

Besides the ordinary nucleotides, the acid extract from E. coli cells was found to contain nucleotide-peptide complexes incorporating one or two nucleotides and from 5 to 10 amino acids (peaks 2, 5-8, 11, 12). Carbohydrate derivatives of nucleotides were also found: xanthinemonophosphate—galactose (peak 9), and UDP-carbohydrate derivatives (peaks 14-16).

The principal components of the nucleotide pool identified by the method described above were taken from the column in the following order (Figs. 1 and 2): hypoxanthine (peak 1), cytidylic acid, AMP-peptide (peak 2), diphosphopyridine-nucleotide (peak 3), adenylic acid (peak 4), a nucleotide-peptide containing adenine (peak 5), a nucleotide-peptide containing adenine and uracil (peak 6), guanylic acid, uridylic acid, triphosphopyridine—nucleotide, an adenine containing nucleotide-peptide (peaks 7-8), xanthinemonophosphate-galactose, ADP-ribose (peaks 8-9), adenosinediphosphate, a nucleotide-peptide containing adenine and cytosine (peak 10), UDP-peptide (peak 11), guanosine diphosphate, a dinucleotide-peptide (peaks 12-13), UDP-glucose, an unidentified nucleotide (peak 14), uridinediphosphate, UDP derivatives, ATP (peaks 15-16), guanosine triphosphate, and ADP derivatives (peaks 17), unidentified compound (peaks 18-20).

With an increase in the age of the culture, a clear increase was found in the content of free bases and nucleosides determined from the amount of US not adsorbed by the Dowex-1 resin, but left in the filtrate and the washings from the column. Among the US the following were identified: adenine, adenosine, uracil, uridine, guanine, guanosine, cytidine, pyridine-peptide [1], pseudouridine, xanthine, and xanthosine.

The content of free bases and nucleosides as a percentage of the total content of US of the acid-soluble fraction of E. coli in different phases of growth was as follows:

Strain of E. coli	Logarithmic phase of growth	Stationary phase of growth
19	1.8	18.3
613	1.1	30.3

A similar increase in the content of bases and nucleosides was observed during lyophilization and storage of the lyophilized cells. For example, the mean content of these compounds for a 6-h culture of E. coli strain No. 19 was 1.8% of the total number of SU in the acid-soluble fraction from the crude bacterial mass and 18% in the case of thelyophilized mass. For E. coli strain No. 613 in the same phase of growth, these values were 1.1 and 10.6 respectively. However, comparison of the elution curves obtained during separation of the acid-soluble fraction from the lyophilized and crude bacterial mass revealed no significant differences, i.e., the picture of the pool remained constant. This showed that the increase in the content of bases and nucleotides evidently took place as a result of the profound enzymic degradation of the nucleic acids after death of part of the cells during lyophilization or aging ot the culture. The results of measurement of the absorption of the culture fluid in UV light after removal of the bacteria by centrifugation showed a clear increase in absorption at 260 mμ during aging of the culture (Fig. 3). This evidently took place on account of lysis of the nonviable cells and accumulation of disintegration products in the surrounding medium. The appearance of a peak at 260 mμ after growth for 24-48 h corresponded with the decrease in the number of bacterial cells at this time, and measured by the decrease in the optical density of the culture at 500 mμ.

In Figure 1, which gives the elution curves of the acid-soluble nucleotides from the cells of E. coli, strain No. 19, growth in MPB, no marked differences can be observed between the composition of the pool in the stationary and the logarithmic phase of growth. An increase in the content of nucleotide-peptides will be noted in the phase of exponential growth (peaks 2, 6, 11). A tendency toward accumulation of nucleotide–peptides in the young culture was also observed in the synthetic medium (Fig. 2), although in this medium the peaks of the nucleotide-peptides were less clearly marked than in MPB.

The tendency toward accumulation of nucleotide-peptides in the logarithmic phase of growth in a rich nutrient medium, i.e., in conditions of intensive protein synthesis, demonstrates that these compounds are actively incorporated into the process of formation of the macromolecules of the cells de novo, and also, perhaps, in the reutilization of existing material for synthesis.

When the two nutrient media were compared, a marked increase was found in the peaks 13-16 in the acid-soluble fraction of the cells grown in M9 (Figs. 1 and 2). This increase in the remote peaks in M9

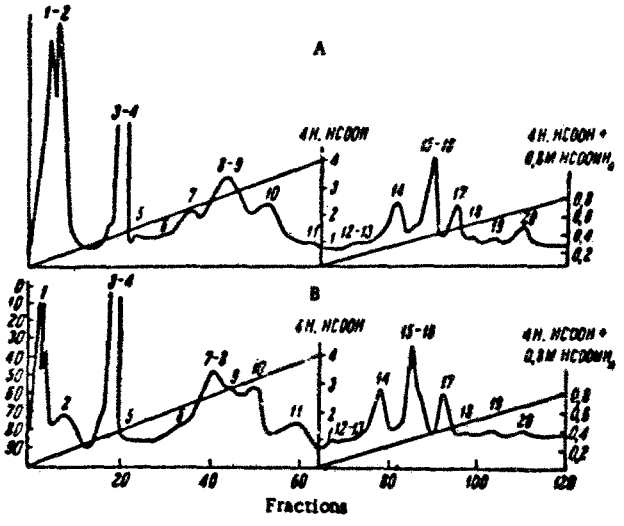

Fig. 2. Separation of free nucleotides from cells of E. coli strain No. 19 cultivated in M9, on a column.

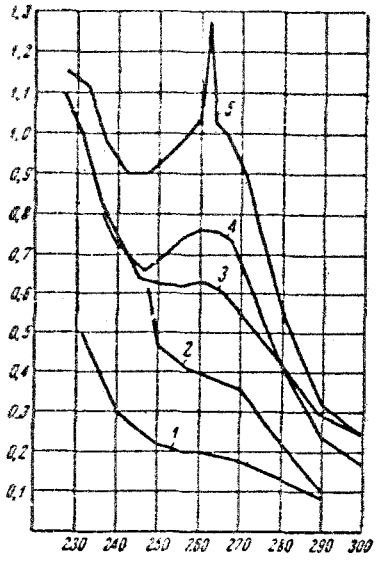

Fig. 3. Excretion of US into the culture fluid during growth of E. coli in M9. Along the axis of abcissas—wavelength; along the axis of ordinates—extinction; 1) 4 h; 2) 6 h; 3) 24 h; 4) 28 h; 5) 46 h.

was observed with both strains of E. coli. Thus both strains of E. coli showed a tendency toward accumulation of UDP derivatives and complication of the picture of the remote peaks during a change from MPB to a medium of simple composition.

The accumulation of uridine derivatives, first discovered during treatment of cells of Staphylococcus aureus with penicillin [8], was subsequently found during treatment of staphylococci with L-cycloserine, oxamycin (D-cycloserine), novobiocin, gentian violet [10, 11], ristocetins [14], and cephalotin [4]. In all these cases a disturbance of one or other stage of synthesis of the cell membrane and accumulation of muramine-peptide derivatives of uridine diphosphate were observed.

Analysis of the ion-exchange chromatograms (Fig. 2) shows that when E. coli is grown in M9, the content, not only of Park's nucleotides, but also of other uridine diphosphate—sugars and also of unidentified components of the remote peaks, increases. The accumulation of UDP derivatives in the cells of E. coli grown in the synthetic medium indicates certain changes in the systems in which UDP derivatives perform the role of coenzymes in the transfer of carbohydrate and carbohydrate-peptide fragments. Possibly in the condicions of a semi-minimal nutrient medium the ability to integrate activated segments into the polymers of the bacterial cell, and especially into bacterial polysaccharides and mucopeptides, is depressed.

The picture of the free nucleotide pool thus accurately reflects the physiological state of the bacterial cell, and the condition of the individual components of this pool may give evidence of the functioning of the more important physiological systems of the cell.

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