Sequential Synthesis of Histidine-degrading Enzymes in Bacillus subtilis*

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EDWARD KAMINSKAS† AND BORIS MAGASANIK
From the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

SUMMARY

L-Histidine induces the formation of the four enzymes constituting the histidine-degrading pathway in Bacillus subtilis. Studies of the kinetics of induction of histidase and of FGA hydrolase demonstrated that the two enzymes appear after induction in sequence, separated by a 2-min interval. The results of experiments in which chloramphenicol was used to arrest peptide bond formation indicate that both enzymes are first formed as enzymatically inactive precursors. The basis for the sequential appearance of these enzymes and of their precursors was shown to be the sequential appearance of their respective enzyme-forming capacities. Experiments using rifampicin, an inhibitor of the initiation of transcription, provide good evidence that the synthesis of messenger RNA specifying both enzymes is initiated upon addition of the inducer. These findings support the hypothesis that the structural genes of the two enzymes are transcribed into a single polycistronic messenger.

Hartwell and Magasanik (1, 2) described the sequence of events leading to the induced synthesis of histidase (L-histidine ammonia lyase, EC 4.3.1.3) in Bacillus subtilis. They showed that the first enzyme molecules appear after a period of 5 min following the addition of histidine, the inducer of the enzyme. The first 30 sec of this period are required by cells to accumulate sufficient histidase to cause induction. At about 2 min following the addition of the inducer the first molecules of the histidase-specific mRNA are completed. This was demonstrated in experiments using actinomycin D, which is taken up by Bacillus subtilis and causes an arrest of DNA-dependent RNA synthesis. When an induced culture was treated with chloramphenicol, which arrests protein synthesis by interfering with peptide bond formation, incorporation of an amino acid into protein stopped immediately, however, histidase continued to accumulate for several minutes. This finding suggested a step in the biosynthetic sequence in which completed peptide chains are converted into active enzyme molecules. This conversion of enzyme precursor to active enzyme was sensitive to low (0°) and high (65°) temperatures, and to treatment of the cells with toluene and with sulfhydryl group inhibitors. The process was not affected by chloramphenicol, puromycin, sodium azide, or oxygen deprivation. It was concluded that it was probably a conformational arrangement of finished peptide chains into an enzymatically active histidase molecule, possibly accompanied by the formation of disulfide bonds. In the biosynthetic time sequence the first histidase precursor molecules appear about 3 min after the addition of the inducer. They are converted into active enzyme molecules in about 2 min.

The capacity for histidase formation was found to be produced at a constant rate from the time of its first appearance at 2 min after addition of the inducer. It decayed with a half-life of 2.4 min and its rate of decay was proportional to its intracellular concentration. A steady state was reached within several minutes after induction and the linear increase in the number of histidase molecules per cell was the expected result of a constant intracellular level of histidase biosynthetic capacity.

Histidase is the enzyme catalyzing the first reaction in histidine degradation in B. subtilis. The subsequent three reactions are catalyzed by urocanase, 4-imidazolone-5-propionate hydrolase, and FGA hydrolase (3-5). These four enzymes are induced by histidine and repressed by catabolites (3, 5). In constitutive mutants all four enzymes are synthesized in the absence of added histidine, but are still subject to catabolite repression. In catabolite-insensitive mutants all four enzymes escape repression by catabolites, but are normally inducible. Therefore, the four histidine-degrading enzymes possess a common gene controlling inducibility and a common gene controlling sensitivity to catabolite repression. The two control genes and the four structural genes are closely linked in the following order: catabolite repression-inducibility-histidase-urocanase-4-imidazolone-5-propionate hydrolase-FGA hydrolase (3, 5).

Development of a sensitive assay for FGA hydrolase (4) provided us with an opportunity to study the molecular events leading to the induced synthesis of this enzyme. Since induced cultures could be assayed for both FGA hydrolase and histidase, the biosynthetic steps in the formation of both enzymes could be compared and the mode of transcription of the two genes examined.

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† Recipient of Postdoctoral Research Scholarship PRS 24 from the American Cancer Society, Inc. Present address, Departments of Medicine, Harvard Medical School and Beth Israel Hospital, Boston, Massachusetts 02115.

The abbreviations used are: FGA, N-formimino-L-glutamic acid; CTB, cetyltrimethylammonium bromide; DOC, sodium deoxycholate.
Experimental Procedure

Chemicals—L-Histidine hydrochloride (Sigma), sodium citrate, 14C-leucine, and 14C-uracil (New England Nuclear Corporation), sodium deoxycholate, cetyltrimethylammonium bromide (Eastman Organic Chemicals), Bio-Rad AG 1-X ion-exchange resin, 200 to 400 mesh, chloride form (Calbiochem), chloramphenicol (Parke, Davis and Company) were obtained commercially. The AG 1 X resin in chloride form was converted to the acetate form. Actinomycin D was a gift from Mr. W. B. Gall of Merck, Sharp and Dohme, Rahway, New Jersey. Rifampicin was a gift from Dr. E. Sensi of Lepetit S.p.A., Milan, Italy. 14C-N-Formimino-L-glutamic acid labeled in the formimino carbon (specific activity 0.001 mCi per minute) was synthesized as described elsewhere (4).

Bacteria—The organism used in this paper, B. subtilis strain SH, is a derivative of SB19. Strain SH is inducible for histidine-degrading enzymes and subject to catabolite repression. Strain SH32, which was derived from SH, is partially constitutive and hyperinducible (3). Strains were stored as spores on potato dextrose agar (special slants at room temperature. Cultures were prepared by inoculating spores into sodium citrate (0.2%), ammonium sulfate (0.2%), salts medium containing 0.01% tryptone (4). Upon germination of spores the culture was diluted at least 250-fold into medium without tryptone. Growth was continued in flasks of 10 times the volume of the culture in a rotary shaker at 37°. The culture was allowed to reach a density giving a reading of 100 to 120 units in a Klett-Summerson photoelectric colorimeter using a No. 42 filter (4×105 Colony-forming units per ml). Doubling time of this organism under these conditions was 140 min. L-Histidine was used as the inducer at a final concentration of 2×10−4 M in all experiments except where noted. Actinomycin D and chloramphenicol were used at final concentrations of 10 μg per ml and 100 μg per ml, respectively. Rifampicin was used at a final concentration of 10 μg per ml.

Enzyme Assays—All assays were performed on cells treated with CTB and DOC (0.01 ml of 0.1% CTB and 0.05 ml 1% DOC per ml of culture). Cells thus treated gave the same enzyme activities as toluenized cells or cells sonically disrupted for 1 min in an MSE sonicator (Instrumentation Associates). The histidase assay was performed as described previously (1).

FGA hydrolyase was assayed by a previously described method adapted for whole cells (4). One milliliter of cells was resuspended in an equal volume of cold 0.05 M Tris-acetate buffer, pH 8.70, containing 5×10−4 M MnCl₂, treated with 0.10 ml of 0.1% CTB and 0.05 ml of 1% DOC, and kept in an ice bath until assay. The reaction was started by the addition of 0.05 ml of 0.20 M 14C-FGA to the cell suspension previously incubated for 10 min at 37°. It was stopped with 0.2 ml of 2% formaldehyde and removal to an ice bath. A 0.70-ml aliquot of the reaction mixture was then loaded on an AG 1-X acetate column made from a shortened Pasteur pipette plugged with glass wool and containing 0.5 ml of the resin. A product of the reaction, 14C-formamide, was eluted with two washes of water (0.5 ml and 1.0 ml) and the effluent collected into two scintillation vials. Twenty milliliters of Bray's solution were added to each vial and the radioactivity in both vials was measured in a Nuclear Chicago Mark I scintillation counter. FGA hydrolyase converts 1 mole of 14C-FGA into 1 mole of 14C-formamide and 1 mole of glutamic acid. Since 14C-FGA is unstable at pH 8.70 (half-life at 37° is 76 hours), it was necessary to run a reaction mixture omitting the cell preparation. Radioactivity of the eluate from this reaction mixture was subtracted as due to nonenzymatic hydrolysis from the counts in each set of eluates from reaction mixtures containing treated cells. The lowest rates of enzymatic hydrolysis (in preparations of uninduced cells) were at least twice the rate of nonenzymatic hydrolysis. The reaction was proportional to the concentration of treated cells in the reaction mixture and to the time of incubation for at least 4 hours, provided not more than 15% of the substrate was hydrolyzed (Fig. 1). One unit of the enzyme is defined as producing 1 μmole of 14C-formamide per min. Because of the lower substrate concentration in the reaction mixture the activity obtained with this assay corresponds to 13.3% of the activity obtained with the cell extract assay (4).

Cell Sampling Procedures—In experiments in which the actual enzyme activity present in the cells at the time of sampling was measured, two methods were used to sample cells. In the first, 2 ml of culture were placed into ice-cold test tubes containing...
0.2 ml of 0.1% CTB and 0.1 ml of 1% DOC and shaken vigorously for 5 sec. The cells were collected by centrifugation at 4340 × g for 10 min and resuspended in 2 ml of cold 0.05 mM Tris-acetate, pH 8.70, 5 × 10⁻⁴ M MnCl₂ buffer. CTB (0.2 ml of 0.1% solution) and DOC (0.1 ml of 1% solution) were added; 1.15 ml of the cell suspension was used for the FGA hydrolase assay and 0.575 ml for the histidase assay. In the second method, 2 ml of culture was sampled into 20 ml of the above Tris-acetate buffer at -2°C. Cells were collected by centrifugation, resuspended in 2 ml of the buffer, and then treated with CTB-DOC. Both methods gave the same results. There was no loss of enzyme activity, unless the buffer solution into which the cells had been sampled froze. Both methods are effective in stopping protein synthesis, measured as ¹⁴C-leucine incorporation into hot trichloroacetic acid-precipitable material, as treatment of cells with cold 10% trichloroacetic acid.

In experiments in which cells were treated with actinomycin D, chloramphenicol, or rifampicin, 2-ml samples were incubated in the presence of the drug at 37°C for 30 min, this time being sufficient for conversion of all enzyme-forming capacity into the active enzyme. At the end of incubation the cells were collected by centrifugation, resuspended in 2 ml of the Tris-acetate buffer, and treated with CTB-DOC.

In dilution experiments a large volume of cells was grown to a Klett reading of 90 to 100. A 50-ml portion of the culture to be used for induction was removed and allowed to continue growth, while the rest was centrifuged to obtain conditioned medium for dilution of the culture (2). The cells to be induced were collected by centrifugation at 15,000 × g for 10 min and resuspended in 2 ml of cold 0.05 mM Tris-acetate, pH 8.70, 5 × 10⁻⁴ M MnCl₂ buffer. CTB (0.2 ml of 0.1% solution) and DOC (0.1 ml of 1% solution) were added; 1.15 ml of the cell suspension was used for the FGA hydrolase assay and 0.575 ml for the histidase assay. In the second method, 2 ml of culture was sampled into 20 ml of the above Tris-acetate buffer at -2°C. Cells were collected by centrifugation, resuspended in 2 ml of the buffer, and then treated with CTB-DOC. Both methods gave the same results. There was no loss of enzyme activity, unless the buffer solution into which the cells had been sampled froze. Both methods are effective in stopping protein synthesis, measured as ¹⁴C-leucine incorporation into hot trichloroacetic acid-precipitable material, as treatment of cells with cold 10% trichloroacetic acid.

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In experiments studying the effects of dilution, 0.5 ml of a 10-fold concentrated culture was diluted into 49.5 ml of prewarmed conditioned medium in a 500-ml flask. The culture was incubated in a rotary shaker at 37°C and 5-ml samples were transferred to test tubes containing chloramphenicol. The cells were allowed to incubate in the presence of the drug for 30 min at 37°C. They were then collected by centrifugation and resuspended in 0.5 ml of the same medium, treated with CTB-DOC, and assayed for histidase. Rates of ¹⁴C-uracil incorporation into RNA were determined by incubating the diluted cultures with ¹⁴C-uracil (0.1 μCi per 0.1 mg per ml of culture). Samples of 5 ml were taken at intervals and pipetted into 2.5 ml of cold 15% trichloroacetic acid. The suspensions were filtered through Millipore AA filters, and washed with 25 ml of cold 5% trichloroacetic acid. The filters were dried and placed into scintillation vials; 20 ml of Bray's solution was added and the radioactivity determined in a Nuclear Chicago Mark I scintillation counter. Rates of ¹⁴C-leucine incorporation into protein were determined by incubating the cells, preinduced for leucine uptake with 0.01 mg of leucine per ml of culture, with ¹⁴C-leucine (0.1 μCi per 0.015 mg per ml of culture). Samples of 5 ml were taken at intervals and pipetted into 2.5 ml of cold 15% trichloroacetic acid. The suspensions were boiled for 30 min, cooled, filtered through Millipore filters, and washed with 25 ml of cold 5% trichloroacetic acid containing 5 μg per ml of leucine. The radioactivity in each filter was determined in a scintillation counter (as above).

Studies of uptake and release of ¹⁴C-histidine by chloramphenicol-treated cells were carried out as described by Hartwell and Magasanik (2).

RESULTS

Induction of FGA Hydrolase—Exponentially growing cultures of B. subtilis were induced with 2 × 10⁻⁴ M histidine, and assayed for FGA hydrolase at intervals. From previous work it was known that treatment of the cells with toluene or suspension of cells in medium at 0°C does not lead to loss of active enzyme but prevents the conversion of histidase precursor into active histidase (2). For this reason the cells to be assayed for FGA hydrolase were either treated immediately with CTB-DOC and placed in an ice bath or diluted into 10 volumes of Tris-acetate buffer at -2°C. Values for FGA hydrolase activity were the same with either procedure. Fig. 2 demonstrates that after the addition of histidine to the culture, FGA hydrolase activity remains at the unduced level for 7 min, then begins to rise, the rate of increase becoming maximal at about 9 to 10 min after induction.

Alternate samples of the same culture were treated with chloramphenicol and incubated at 37°C for 30 min in order that full conversion of a putative FGA hydrolase precursor into active enzyme would take place. As Fig. 2 demonstrates, an FGA hydrolase precursor begins to form at about 4½ to 5 min following induction. From the two induction curves it can be estimated that the conversion of an FGA hydrolase precursor into an active enzyme molecule takes about 2 min.

Synthesis of Histidase and of FGA Hydrolase—FGA hydrolase activity appeared consistently later than histidase activity in induced cells. Fig. 3 demonstrates this relationship. Histidase activity starts to rise at about 5½ min after induction and FGA hydrolase activity at 7 min.

This sequential appearance was more fully investigated in

![Fig. 2. The formation of FGA hydrolase and FGA hydrolase precursor after the addition of histidine to the growth medium. An exponentially growing culture (5 × 10⁸ cells per ml) was induced with 2 × 10⁻⁴ M histidine and 1-ml samples taken at times indicated were either immediately treated with CTB-DOC and placed in an ice bath (O—O) or treated with chloramphenicol and incubated at 37°C for 30 min (0—0).](http://www.jbc.org/issue/574/14/3551/fig2.jpg)
experiments in which actinomycin D was used to arrest RNA synthesis and chloramphenicol to arrest protein synthesis. Cells sampled into actinomycin D and incubated until enzyme accumulation has come to a halt contain the enzyme activity proportional to the number of enzyme-specific mRNA chains completely transcribed at the time of sampling. Thus each point on the induction curve of samples treated with actinomycin D represents the enzyme-forming capacity of the culture at the time of sampling. Cells sampled into chloramphenicol and similarly incubated contain the enzyme activity proportional to the total number of enzyme-specific mRNA chains completely translated at the time of sampling. In these experiments an exponentially growing culture was divided into two flasks. Samples from one flask were treated with actinomycin D and from the other with chloramphenicol. All the samples were incubated at 37° for 30 min, this time being sufficient for the conversion of all precursor molecules into active enzyme molecules. A composite graph made from both sets of data (Fig. 4)

FIG. 3. The formation of histidase and of FGA hydrolase after the addition of histidine to the growth medium. An exponentially growing culture (5 x 10⁸ cells per ml) was induced with 2 x 10⁻⁶ M histidine. At times indicated 1-ml samples were treated with CTB-DOC and assayed for either histidase activity (Δ—Δ) or FGA hydrolase activity (O—O).

FIG. 4. The appearance of histidase- and of FGA hydrolase-forming capacities after induction. An exponentially growing culture (5 x 10⁸ cells per ml) was divided into two portions and each was induced with 2 x 10⁻⁴ M histidine. Samples from one were treated with actinomycin D and from the other with chloramphenicol and were incubated at 37° for 30 min. Each sample was assayed for histidase and FGA hydrolase activities. Histidase (Δ—Δ) and FGA hydrolase (O—O) in actinomycin D-treated cells; histidase (Δ—Δ) and FGA hydrolase (O—O) in chloramphenicol-treated cells.

FIG. 5. The appearance of cumulative histidase- and FGA hydrolase-forming capacities after induction. An exponentially growing culture (4 x 10⁸ cells per ml) was induced with 2 x 10⁻⁴ M histidine. At times indicated, 2 ml samples were removed and treated either with rifampicin or with actinomycin D and allowed to incubate at 37° for 30 min. Histidase (Δ—Δ) and FGA hydrolase (O—O) in actinomycin D-treated cells; histidase (Δ—Δ) and FGA hydrolase (O—O) in actinomycin D-treated cells. Arrow (†) indicates the time of addition of histidine.

indicates that the first completed messages for histidase appear at about 2 min and for FGA hydrolase at about 3 ½ min following induction. Histidase precursor molecules first appear at about 3 min and FGA hydrolase precursor molecules at about 5 min following induction. Therefore, the earlier appearance of histidase in induced cells is due to the earlier transcription and translation of the mRNA specific for histidase.
actinomycin D-treated samples, the two enzymes appear in the sequence described in the previous section. This plot of enzyme-forming capacities clearly shows that the initiation of transcription of both messages takes place at the same time, soon after induction.

**Effect of Inducer on Induction of Histidase and FGA Hydrolase**—The role of the inducer in the synthesis of inducible enzyme can be examined by experiments in which the inducer is removed at appropriate intervals after induction (2, 8, 9). Previous studies have shown that *B. subtilis* W23G cells rapidly take up added histidine. Within 30 sec the cells accumulate histidine at a level sufficient for full induction. On dilution of cells with histidine-free conditioned medium the intracellular histidine concentration drops quickly to levels insufficient for induction and synthesis of histidase ceases (2). The following experiments were performed in order to compare the effect of inducer removal on induction of the two enzymes.

A preliminary experiment determined the inducing levels of histidine for SH cells. This strain requires \(1 \times 10^{-4} \text{M} \) histidine for full induction. On the other hand, for induction not to occur, histidine concentration has to be \(5 \times 10^{-7} \text{M} \) or lower. These findings differ markedly from those in strain W23G, in which induction is complete at \(5 \times 10^{-5} \text{M} \) and does not occur at the \(5 \times 10^{-4} \text{M} \) concentration. Because a 200-fold dilution is required to lower the concentration of histidine in the medium below inducing levels, in the following experiments the cells to be induced were first collected by centrifugation and then resuspended in 0.10 volume of the same medium. Following induction with \(1 \times 10^{-4} \text{M} \) histidine samples were diluted 200-fold in conditioned medium and were incubated at \(37^\circ \text{C} \) for 30 min in order that all the induced enzyme-forming capacities be completely expressed. Control experiments show that a culture concentrated 10-fold in this manner synthesized protein, RNA, and induced histidase equally well as an unconcentrated culture.

Inducer dilution experiments were carried out by sampling a culture induced with \(1 \times 10^{-4} \text{M} \) histidine alternately into 200 volumes of histidine-free conditioned medium or into 9 volumes of conditioned medium containing actinomycin D. The cells were incubated with shaking at \(37^\circ \text{C} \) for 30 min and were then collected by centrifugation and assayed for both enzymes. Fig. 6 shows the results to be very similar to those obtained by use of rifampicin; histidase and FGA hydrolase activities rose simultaneously soon after induction in diluted samples, whereas, in actinomycin D-treated samples they appeared sequentially. These results are consistent with the postulate that the initiation of transcription of both messages takes place at the same time soon after induction. In addition, they were interpreted to mean that continued presence of the inducer is not necessary for transcription of initiated messages to continue.

Interestingly, 20-fold dilutions of the samples gave the same results as 200-fold dilutions, but 10-fold dilutions yielded results suggesting a constant limited amount of reinitiation; the enzyme activities were displaced upward and the base line intersected to the left. Since a 20-fold dilution of a sample would lower the internal histidine concentration to only \(5 \times 10^{-8} \text{M} \), these results implied that other factors besides the internal concentration of histidine affect induced enzyme synthesis in the diluted samples. Indeed, it became clear that dilution itself stops induced formation of histidase and that presence or absence of histidine in the diluting medium is not important. Fig. 7 shows the results of an experiment in which the culture was concentrated and then induced...
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FIG. 8. Effect of a 100-fold dilution on an uninduced and an induced culture. A portion of an exponentially growing culture was concentrated 10-fold by centrifugation and resuspension in 0.10 volume of the same medium. A 0.5-ml portion of this concentrated culture was diluted into 49.5 ml of conditioned medium. The normally growing, the concentrated, and the diluted portions of the culture were induced with $5 \times 10^{-5} \text{M}$ histidine. At 5 min after induction, 0.5 ml of the concentrated culture was diluted into 49.5 ml of conditioned medium containing $5 \times 10^{-5} \text{M}$ histidine (†). Samples of 0.5 ml from the undisturbed culture and of 5 ml from the diluted cultures were treated with chloramphenicol and were incubated at 37°C for 30 min. Each point represents histidase activity per sample of cells, expressed as histidase activity per ml of the usual experimental culture (unconcentrated and undiluted). Histidase in a normally growing induced culture (O---O); histidase in a diluted, then induced culture (O- - -O); histidase in an induced, then diluted culture (X---X).

FIG. 9. Effect of a 100-fold dilution of an induced culture on the rates of protein and RNA synthesis. An exponentially growing culture was collected by centrifugation and resuspended in 0.10 volume of the same medium. One portion of the culture was induced with $5 \times 10^{-5} \text{M}$ histidine 5 min before dilution. A volume of 0.5 ml of the induced culture was added to 49.5 ml of conditioned medium with or without $5 \times 10^{-5} \text{M}$ histidine and containing $^{14}$C-uracil (A) or $^{14}$C-leucine (B). Samples (5 ml) from each culture flask were pipetted into 2.5 ml of cold 15% trichloracetic acid and processed as described under "Experimental Procedure." Each point represents counts per min incorporated per 5 ml of cells in an uninduced diluted culture (A-----A); in induced culture diluted into histidine-containing medium (O-----O); in induced culture diluted into histidine-free medium (X-----X).

Chloramphenicol-treated SH cells took up histidine equally as well as similarly treated W23G cells, but they completely failed to release it during a period of 30 min following a 200-fold dilution with histidine-free conditioned medium.

These results lead us to postulate that it is the dilution itself that affects the induced synthesis of histidase. In a further study the sequence of induction and dilution proved to be important (Fig. 8). When the culture was first diluted, then induced with $5 \times 10^{-5} \text{M}$ histidine, there was a lag of about 10 min in the appearance of the active enzyme, followed by a constantly increasing rate of enzyme synthesis, so that 40 min after induction the culture had acquired 84% of the enzyme activity of the undisturbed culture. When, in the same experiment, the cells were first induced, then diluted 100-fold into histidine-containing medium, histidase was synthesized at a very low rate, so that by 40 min after induction the culture had less than 20% of the enzyme activity of the control culture. That amount of activity would be expected from the cumulative enzyme-forming capacity present in the cells at the time of dilution. Yet, the rates of protein and RNA synthesis, as measured by incorporation of
\(^{14}\)C-leucine into hot trichloroacetic acid precipitable material and of \(^{14}\)C-uracil into cold trichloroacetic acid precipitable material, were the same in an uninduced diluted culture and in cultures first induced, then diluted either into histidine-free or into histidine-containing medium (Fig. 9).

Constitutive synthesis of histidase in strain SH32 was found to be unaffected by a 100-fold dilution with conditioned medium. Apparently, dilution makes the cells unresponsive to induction by histidine.

**DISCUSSION**

We have found that the accumulation of active FGA hydrolase which follows addition of histidine to a culture of \(B.\ subtilis\) is preceded by the accumulation of material that can be converted to the active enzyme when peptide bond synthesis is prevented by chloramphenicol. Apparently, FGA hydrolase, as well as histidase in \(B.\ subtilis\) (1) and \(\beta\)-galactosidase and tryptophanase in \(E.\ coli\) (10, 11), is first synthesized as an enzymatically inactive precursor.

Following the addition of the inducer, accumulation of histidase and of FGA hydrolase molecules proceeds for a short period at steadily increasing rates until a maximal steady state is reached. The kinetics of this process was first analyzed by Hartwell and Magasanik (1), who showed that the steady state linear rate resulted from a constant intracellular concentration by histidine. Apparently, dilution makes the cells unresponsive to induction to be unaffected by a 100-fold dilution with conditioned medium.

Fig. 9.

Constitutive synthesis of histidase in strain SH32 was found to be unaffected by a 100-fold dilution with conditioned medium. Apparently, dilution makes the cells unresponsive to induction by histidine. We cannot exclude this possibility, although the molecular weights of the two enzymes are similar.

According to the second hypothesis, a single mRNA chain specifies both enzymes; the segment specifying histidase is synthesized before the segment specifying FGA hydrolase. A similar situation is well supported by experimental evidence for the \(trp\) operon of \(E.\ coli\) (15, 16). This hypothesis is in good accord with the observation that the control gene determining the inducibility of the histidine-degrading enzymes, the structural gene for histidase, and the structural gene for FGA hydrolase are linked in this order on the \(B.\ subtilis\) chromosome (3). Transcription would begin near the control gene and proceed through the histidase gene to the FGA hydrolase gene. This hypothesis is in good accord with the evidence presented in the preceding paper for the view that the genes affecting histidine degradation constitute an operon (5). The finding that the genes for urocarnase and imidazolonepropionate hydrolase are located between the genes for histidase and FGA hydrolase (5) suggests that the mRNA segments specifying urocarnase and imidazolonepropionate hydrolase are transcribed later than the segment specifying histidase, but earlier than the segment specifying FGA hydrolase. Unfortunately, the insensitivity of the assays for urocarnase and imidazolonepropionate hydrolase precludes verification of this prediction.

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Edvardas Kaminskas and Boris Magasanik


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