ENZYME INDUCTION IN NUTRITIONAL MUTANTS IN PRESENCE OF 5-AMINO-2,4-BIS(SUBSTITUTED-AMINO)PYRIMIDINES

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In a previous paper (Kunkee, 1960a) the formation of induced enzyme in Escherichia coli was shown to be stimulated in the presence of certain 5-amino-2,4-bis(substituted-amino)pyrimidines. In order to determine at which point in the induction mechanism the stimulation occurred, the effects of the stimulator on various nutritional mutants of E. coli were determined.

MATERIALS AND METHODS

Enzyme induction was determined by measuring the formation of β-galactosidase in E. coli. Unless otherwise stated, the materials and methods were the same as given previously (Kunkee, 1960a).

Nutritional mutants of E. coli. We are grateful to Professor A. B. Pardee, University of California (Berkeley), for providing the pyrimidine-requiring mutant (6386) of E. coli (isolated by Professor B. D. Davis), and to Professor S. E. Luria, Massachusetts Institute of Technology, for the thymine-requiring mutant (15T-) E. coli (isolated by Professor J. S. Gots). The tryptophan- and leucine-requiring mutants of E. coli (designated A44 and A46, respectively) were provided by Dr. Dewey H. Smith and Mrs. Sonia S. Sloan of this department. These mutants were prepared by ultraviolet irradiation and isolated using the penicillin technique of Adelberg and Myers (1953). All cultures were maintained on nutrient agar slants. When strain 15T- was used, inoculations of subcultures were made from colonies picked from streaked nutrient agar plates.

For experiments with nutritional mutants, 100 ml of salts medium with glycerol and the required nutrient were inoculated with sufficient (10 to 12 ml) overnight subculture to give ~2 × 10^8 cells per ml, as measured by turbidity (Kunkee, 1960a). The inoculated medium was then shaken for about 2 hr at 37˚C, at which time the cells were in logarithmic growth phase. The cultures were then centrifuged, washed with chilled salts medium, and resuspended in salts medium to give a concentration of ~8 × 10^8 cells per ml. The cells were used immediately for induction experiments as previously described (Kunkee, 1960a). Inoculations were always made into minimal medium also, to check for reversion of mutants to wild type.

For experiments with sulfur deficiency, E. coli strain ML was grown in glycerol salts medium. The cells were then washed and resuspended in salts medium lacking sulfate (and lacking the equivalent amount of potassium). For induction, various amounts of 8 × 10^-5 M K_2SO_4 were added to the induction flasks.

Unless otherwise stated, the stimulator was 5-amino-2,3-bis(2-thienylamino)pyrimidine-acetic acid at a final concentration of 15 μg per ml.

RESULTS

Induction in uracil-less E. Coli. Pardee (1954) has shown that in the presence of an energy source, enzyme induction does not occur in strain 6386 (uracil-less) in the absence of uracil. This work suggests that formation of ribonucleic acid (RNA) is essential for enzyme induction. We have found that in the presence of the stimulator, β-galactosidase induction was stimulated in strain 6386 (uracil-less) growing on glycerol as an energy source and on a limited amount of uracil (figure 1). The stimulator had no effect unless uracil was present, thus the stimulator did not substitute for uracil. This stimulation of induction in the presence of uracil, where uracil is the limiting step, showed that the locus of stimulation is at or beyond the point of utilization of uracil in the induction process. We have shown...
that total RNA synthesis is not increased in the presence of the stimulator (Kunkee, 1960a). However, the stimulator could be preventing the utilization of uracil for reactions other than enzyme induction, and thus allowing a preferential utilization of uracil for the synthesis of specific RNA required for formation of the particular enzyme.

In the absence of an energy source, the uracil requirement for induction in uracil-requiring E. coli disappears (Pardee, 1955). Presumably, under these conditions, where no growth is occurring, there is sufficient breakdown of endogenous nucleic acid to provide for the uracil requirements for induction, and the cell uses the uracil preferentially for these requirements. We found there was no increase of induction by the stimulator in the absence of energy source and uracil (figure 1). One explanation for this result is that all the available uracil is being preferentially directed toward the induction process already, and thus the addition of the stimulator cannot augment this preferential utilization.

In addition, we found there was no stimulation of induction in the uracil-deficient mutant in absence of an energy source and uracil even at low concentrations of inducer (5 to 50 × 10⁻⁵ M melibiose). That is, a situation was obtained where induction could be increased by addition of more inducer but not by addition of stimulator. Thus the action of the stimulator is not directly involved with the inducer. This is further indication that the stimulator is not acting to increase the permeability of the cell to the inducer (Kunkee, 1960a).

**Amino acid-less bacteria.** In amino acid-requiring mutants, the amount of induced enzyme formation is dependent upon the concentration of the required amino acid. Induction in amino acid-less mutants on limiting amounts of the required amino acid was found to be increased in the presence of the stimulator. In figure 2 is shown the stimulation of induction in tryptophan- and leucine-requiring strains of E. coli at low concentrations of the required amino acid. Furthermore, induction in wild-type E. coli in the presence of low levels of sulfate, which would limit the formation of sulfur-containing amino acids, was also increased by the stimulator (figure 2). Again, these results can be explained as a preferential utilization of the amino acids for the formation of the specific enzyme. The stimulator may be causing this preferential utilization by inhibiting other reactions involving the amino acids, or by causing a preferred syn-

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**Figure 1.** β-Galactosidase induction in Escherichia coli strain 6986 (uracil-less), with 0.013 M melibiose as inducer. In the graph on the left, induction was carried out in the presence of an energy source (0.4 per cent v/v glycerol) and a limiting amount (14 μg per ml) of the required nutrient, uracil. In the graph on the right, both glycerol and uracil were lacking. (○) Control, (●) 5-amino-2,4-bis(2-thylenamino)pyrimidine-acetic acid, 15 μg per ml.

**Figure 2.** β-Galactosidase induction with limiting levels of amino acids. Induction was carried out in indicated strains of Escherichia coli using 0.013 M melibiose as inducer. (○) Control, (●) 5-amino-2,4-bis(2-thylenamino)pyrimidine-acetic acid, 15 μg per ml. For the sulfur-deficient experiment, graph on the far right, the stimulator, 5-amino-2,4-bis(furfurylamino)pyrimidine, which contains no sulfur, was used (64 μg per ml) (▲).
thesis of the protein forming system for this particular enzyme.

It has been suggested that vitamin B₁₂ is involved in protein biosynthesis (Wagle et al., 1958). We found that vitamin B₁₂ had no significant stimulatory effect on the induction of β-galactosidase in wild E. coli. However, when enzyme induction was carried out in tryptophan-requiring E. coli with limiting amounts of tryptophan, vitamin B₁₂ gave no stimulation, but produced an additional increase in induction in the presence of the stimulator (figure 3). In the absence of the stimulator, B₁₂ was ineffective. These results suggest a relationship between the mechanism of the stimulation and the activity of B₁₂. In this respect, it is interesting to observe that folic acid, which seems to be related to vitamin B₁₂ in activity, is also in some way involved in the activity of the stimulator; the stimulator has a sparing effect on the folic acid requirement of streptococci (Kunkee, 1960b).

Induction in thymine-less mutants. The effect of the stimulator on induction during thymine deficiency was also studied. Cohen and Barner (1955) have shown that xylose isomerase induction can occur in the absence of thymine in the thymine-requiring (15'T-) bacterium, E. coli. We have found this to be true with β-galactosidase induction also. Stimulation of induction occurred in the mutant both in the presence and in the absence of thymine. The amount of stimulation was not as great as that found with E. coli strains B or ML, but was comparable to that found in the parent strain, E. coli 15 (Kunke, 1960a). These results indicate that there is no direct relationship between the inducer or the stimulator and thymine metabolism in this system.

DISCUSSION

The results presented suggest the following as a working hypothesis for the mechanism of action of the stimulator. The direct action of the stimulator is to reduce in some manner the synthesis of RNA in the various protein-forming systems of the cell, and allow the increased formation of the specific RNA needed for induced enzyme synthesis. On limited amounts of uracil, the stimulation of induction occurs because, in the presence of the stimulator, nearly all of the uracil is being directed toward the formation of the specific RNA material. Also, the stimulation occurs on limited amounts of amino acids because, in the presence of the stimulator, most of the RNA in the protein-forming systems is specific for induced enzyme synthesis, and thus the limited amounts of amino acids are used preferentially for the induced enzyme synthesis.

We can speculate on the mechanism of action of the stimulator which would bring about a preferential synthesis of specific RNA as proposed above. A new theory for the mechanism of enzyme induction has been put forth by Pardee et al. (1958). From their results they have concluded that formation of induced β-galactosidase results from the antagonism of a natural repressor by the enzyme inducer. We have no direct evidence that stimulation of induction by 5-amino-2,4-bis-(2-thienylamino)pyrimidine-acetic acid, 15 μg per ml.

Figure 3. Induction of β-galactosidase in the presence of various concentrations of vitamin B₁₂ in E. coli strain A44 (tryptophan-less). Induction was carried out on limiting levels of L-tryptophan (3 μg per ml), for 30 min, with 0.013 M melibiose as inducer. (O) Control, (●) 5-amino-2,4-bis-(2-thienylamino)pyrimidine-acetic acid, 15 μg per ml.
stimulator cannot be explained on the basis of inhibition of the repressor alone. If the stimulator acts only at some early step in the induction process, there would be no stimulation when one of the final steps were limiting, as occurs during amino acid deficiency.

Other evidence that RNA is involved in the stimulation is presented in another paper (Kunke, 1960b) in which data are given showing a sparing effect by the stimulator on the folic acid requirement of streptococci.

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SUMMARY

Induction of $\beta$-galactosidase in the presence of certain $5$-amino-$2,4$-bis(substituted-amino)pyrimidines was examined using nutritional mutants of Escherichia coli. Induction was stimulated in the presence of the substituted pyrimidines in uracil-less and amino acid-less bacteria on low levels of the required nutrients, and in wild bacteria on low levels of sulfur. In the uracil-less mutants there was no stimulation of induction unless an energy source was present. In thymine-less E. coli, enzyme induction was stimulated by the substituted pyrimidines both in the presence and in the absence of thymine.

It was hypothesized that the stimulatory action occurred by the stimulated formation of the specific ribonucleic acid of the protein-forming system involved in the synthesis of the induced enzyme.

REFERENCES


Kunke, R. E. 1960b Sparing effect on the folic acid requirement of Streptococcus lactis by 5-amino-2,4-bis(substituted-amino)pyrimidines. J. Bacteriol., 79, 55-57.


