ENZYME INDUCTION IN THE PRESENCE OF RIFAMPIN, AN INHIBITOR OF THE TRANSCRIPTION PROCESS

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SUMMARY

Acetylornithine δ-transaminase, an enzyme of the arginine pathway, is inducible by arginine in a mutant strain of Escherichia coli W. Rifampin, an inhibitor of initiation of transcription, does not prevent the induction of this enzyme by arginine, whereas actinomycin D completely eliminates the inductive effect of arginine. Furthermore, although rifampin inhibits the growth of this mutant, it, alone, is just as effective as arginine for the induction of the transaminase. The induction of the transaminase by rifampin, as well as by arginine, requires the presence of a functional regulatory gene for arginine.

The synthesis of all eight enzymes which mediate the biosynthesis of arginine in Escherichia coli strain W is subject to repression by arginine (1,2,3). A mutant of an acetylornithine δ-transaminase (transaminase)-negative strain exhibits an altered control behavior for the transaminase (4). The presence of arginine in the growth medium of this mutant leads to an induced synthesis of the transaminase and a simultaneous repression of synthesis of all other enzymes of the arginine pathway. Regulatory gene mutants ($_{arg}R^-$) of this inducible strain synthesize the arginine-inducible transaminase at very low, noninduced rates even when grown in medium containing arginine, but these mutants produce derepressed levels of all other enzymes in the pathway (4,5,6). These observations suggest that the product of the arginine regulatory gene ($_{arg}R^+$) has a functional role in both induction and repression.

Recent experiments using homoarginine, an analogue of arginine, have indicated that the $_{arg}R^+$ product may govern induction and repression by separate and distinct mechanisms (5). Homoarginine can repress (although less effectively than arginine) the synthesis of acetylornithinase and ornithine transcarbamylase which indicates an interaction between homoarginine and the

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arginine regulatory gene product. Homoarginine does not induce transaminase biosynthesis, but it does delay temporarily the onset of transaminase induction by arginine. These effects of homoarginine are not due to inhibition of arginine transport, to charging of arginyl-tRNA by homoarginine, nor to inhibition of arginyl-tRNA synthetase (5).

In an attempt to elucidate further the mechanisms governing induction and repression of this pathway, experiments were conducted testing the effect of inhibitors known to interfere with the process of transcription. The results presented in this paper indicate that rifampin, unlike actinomycin D, does not interfere with arginine-induced biosynthesis of acetylornithine δ-transaminase. Unexpectedly, however, it was observed that rifampin alone, in the absence of arginine, brings about the induction of the transaminase. Further experiments are currently underway to assess the significance of rifampin-induced synthesis of the transaminase.

MATERIALS AND METHODS

The transaminase-inducible strain used in this study was derived from a transaminase-less strain of E. coli W.

The genotype of the inducible strain is represented by $M^-T^-_{arg}R^+$, referring to the loci for inducibility of the transaminase, the transaminase structural gene, and the arginine regulatory gene, respectively. An arginine regulatory gene mutant ($M^-T^-_{arg}R^-$) of the inducible strain was also used.

The medium of Vogel and Bonner (7) supplemented with L-proline (25 μg per ml) and DL-methionine (100 μg per ml) was used for growth of these strains. Glucose, autoclaved separately, was added aseptically prior to inoculation. L-Arginine was added to the growth medium to give an initial concentration of 100 μg per ml. The actinomycin D studies were carried out using the procedures of Leive (8) except that the concentration of EDTA was 0.02 M and the concentration of actinomycin D was 20 μg per ml. The EDTA treatment alone did not affect the induction of the transaminase. Cell cultures for experiments using rifampin (60 μg per ml) did not require pretreatment with EDTA.

Preparation of cell extracts and enzymatic assays were carried out as previously described (5). Standard assay conditions for acetylornithine δ-transaminase were those described by Albrecht and Vogel (9). Protein was determined, using appropriate buffer blanks, by the method of Lowry et al. (10). Actinomycin D, rifampin, 5-^{3}H-uridine, and α-N-acetyl-L-ornithine were obtained from Calbiochem. o-Aminobenzaldehyde was a product of K & K Laboratories, Plainview, New York. All other chemicals were reagent grade.

RESULTS AND DISCUSSION

Transaminase activity remains at a basal level when the inducible strain

Table I. Induction of Acetylornithine δ-Transaminase Synthesis in E. coli W by Arginine and Rifampin.

The inducible strain ($M^-T^-_{arg}R^+$) and a regulatory gene mutant ($M^-T^-_{arg}R^-$), grown in minimal medium without shaking, were harvested in early log phase and resuspended in minimal medium. The resuspended cells were used to inoculate minimal medium or minimal medium containing rifampin (60 μg per ml). Arginine was added 5 minutes after inoculation at an initial concentration in the growth medium of 100 μg per ml. After incubation for 2 hours, the cells were removed by centrifugation, resuspended in phosphate buffer, and sonically disrupted for transaminase assay. The data presented are averages of ten experiments.

Genetic Constitution	Additions to Medium	Specific Activity
		units/mg protein
$M^-T^-_{arg}R^+$	None	2.5
	Arginine	7.2
	Arginine plus rifampin	8.5
	Rifampin	6.1
$M^-T^-_{arg}R^-$	None	0.8
	Arginine	0.9
	Arginine plus rifampin	0.7
	Rifampin	0.6

is grown in minimal medium (Table I). Addition of arginine results (within 2 minutes) in a rapid burst of synthesis of the inducible acetylornithine δ-transaminase (5). Rifampin, an inhibitor of initiation of transcription (11), at a concentration sufficient for inhibiting growth, does not prevent the rapid onset of induction of the transaminase upon addition of arginine (Figure 1). These results suggest that de novo synthesis of transaminase-specific mRNA is not necessary for this induction process as had been proposed previously (5).

Evidence for regulation at the translational level has been reviewed by Vogel and Vogel (12). Additional support proposed for translational control comes from McLelland and Vogel (13) who have shown recently that the addition of arginine to an E. coli mutant, grown under arginine-restrictive conditions, in the presence of rifampin, does not result in the repression of synthesis of ornithine transcarbamylase. Their proposals are based upon the assumption that specific mRNA is synthesized during arginine restriction but remains cryptic (untranslated) and hence accumulates in the cell. Among alternative hypothesis consistent with their data, one might envision the partial synthesis of specific mRNA, which precludes translation until transcription is completed. Control may therefore occur at the transcriptional level by affecting termination of RNA synthesis and release from DNA, or at the translational level by per-

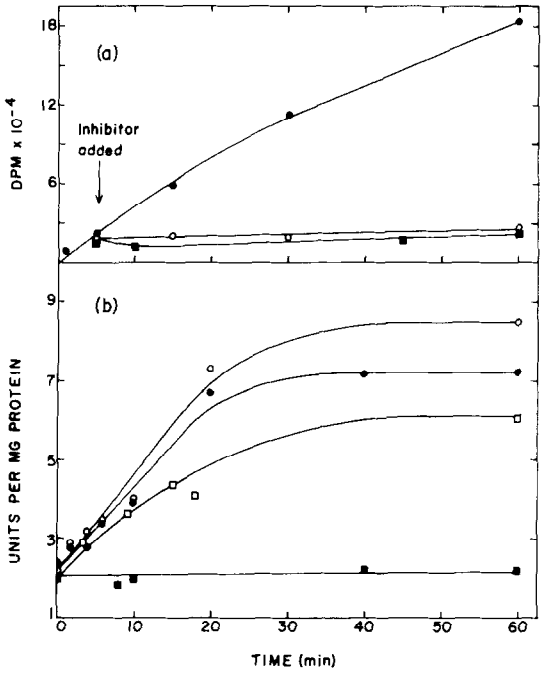

Figure 1. (a) Incorporation of 5-^{3}H-uridine into RNA. E. coli cells from an early log phase culture were harvested and resuspended at a density of 5 x 10^9 cells per ml for EDTA treatment as described in methods. The EDTA treated cells were diluted with amino acid supplemented growth medium E (see methods) and tritiated uridine (2.5 μCi; sp. act., 1 mCi/mmole) was immediately added (0 time on graph). After 5 minutes incubation, the treated culture was divided into two equal portions: to one flask rifampin (60 μg/ml final concentration) or actinomycin D (20 μg/ml final concentration) was added; the other served as the control. Aliquots (0.5 ml) were removed at various time intervals and added to an equal volume of cold 10% TCA. After standing at 4° for 20 minutes, the samples were poured onto millipore filters and washed with 20 ml of cold 5% TCA. The filters were combusted in a Packard Tricarb sample oxidizer, the radioactivity collected as ^3H_2O, and counted in a Packard Tricarb liquid scintillation spectrometer. Quench corrections were made for conversions to DPM. The data represent an average of duplicate samples from each of two experiments.
(b) Effect of rifampin and actinomycin D on the rate of synthesis of arginine-induced acetylornithine δ-transaminase. Arginine (100 μg/ml) was added 5 minutes after the addition of rifampin or actinomycin D (concentrations same as 1a). Fractions were removed at various time intervals; after all samples had been collected, the cells were removed by centrifugation, resuspended in phosphate buffer, and sonically disrupted for enzymatic assay (5). These data represent an average of ten experiments. Symbols for 1a and 1b: ●, arginine; O, arginine plus rifampin; □, rifampin; ■, arginine plus actinomycin D.

mitting ribosomes to begin to translate the partially synthesized mRNA, which, in turn, promotes completion of transcription (translational mediated transcription).

Our evidence appears to be consistent with a translational mediation of transcription of the inducible transaminase in this mutant, although the possibility of transcriptional control is by no means eliminated.

If the cryptic messenger hypothesis were to constitute the explanation of transaminase induction, then one would predict that the addition of actinomycin D under arginine-restrictive conditions would not prevent induction. Actinomycin D, added 5 minutes prior to the addition of arginine, caused immediate cessation of incorporation of uridine into RNA and completely eliminated induction of the transaminase (Figure 1).

If induction depended upon initiation of synthesis of specific mRNA, one would predict similar results with rifampin. But rifampin, added 5 minutes prior to the addition of arginine, did not affect the kinetics of the onset of transaminase induction by arginine although the incorporation of tritiated uridine into RNA was inhibited approximately 99% (Figure 1). Surprisingly, we have observed that rifampin alone was capable of inducing the transaminase at a rate similar to that of arginine (Figure 1). (Differences in specific activity after 60 minutes may be a reflection of the differential rates of overall protein synthesis versus transaminase synthesis--Table I.) That the induction of transaminase by rifampin involves a specific regulatory control mechanism, is suggested by the necessity for a functional arginine regulatory gene, $_{arg}R^{+}$ (Table I).

In order for the $_{arg}R^{+}$ gene to control transaminase induction (and possibly repression of the other enzymes of arginine biosynthesis) at the translational level, one would have to postulate an interaction between the ribosome and the $_{arg}R$ gene product. Recently, Udaka has isolated a complex in which the $_{arg}R$ gene product is associated with the 30 S ribosomes (14). Thus, the binding of arginine or an arginine derivative by such a complex would alter its configuration to permit initiation or completion of translation, simultaneously facilitating completion (but not initiation) of transcription.

One would expect that the evidence for the facilitation of induction by rifampin alone would argue for control at the transcriptional level since rifampin is known to interact with RNA polymerase. Recently, however, rifampin has been shown to cause ribosomal alterations (15); thus, the possibility exists that transaminase induction in the presence of rifampin occurs as a result of rifampin interacting with the $_{arg}R$ gene-ribosome complex to produce alterations similar to those brought about by arginine. Control at the translational level would also account for the arginine-mediated burst of synthesis of ornithine transcarbamylase (16) and acetylornithinase (5) that is observed before the onset of repression of these enzymes.

It should be pointed out, however, that the ability of rifampin to

facilitate transaminase induction may be peculiar to this system. We are currently investigating the kinetics of repression of other enzymes of the arginine pathway in the presence of rifampin, actinomycin D, and other inhibitors. These studies should help to clarify the mechanism of transaminase induction in this organism.

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REFERENCES

1. Vogel, H. J., Cold Spring Harbor Symp. Quant. Biol., 26, 163 (1961).
2. Gorini, L., Gundersen, W., and Burger, M., Cold Spring Harbor Symp. Quant. Biol., 26, 173 (1961).
3. Maas, W. K., Cold Spring Harbor Symp. Quant. Biol., 26, 183 (1961).
4. Bacon, D. F., and Vogel, H. J., Cold Spring Harbor Symp. Quant. Biol., 28, 473 (1963).
5. Forsyth, G. W., Carnevale, H. N., and Jones, E. E., J. Biol. Chem., 244, 5226 (1969).
6. Theil, E. C., Forsyth, G. W., and Jones, E. E., J. Bacteriol., 99, 269 (1969).
7. Vogel, H. J., and Bonner, D. M., J. Biol. Chem., 218, 97 (1956).
8. Leive, L., Biochem. Biophys. Res. Commun., 18, 13 (1965).
9. Albrecht, A. M., and Vogel, H. J., J. Biol. Chem., 239, 1872 (1964).
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem., 193, 265 (1951).
11. Wehrli, W., Knusel, F., Schmid, K., and Staehlin, M., Proc. Nat. Acad. Sci. U. S. A., 61, 667 (1968).
12. Vogel, H. J., and Vogel, R. H., Ann. Rev. Biochem., 36, 519 (1967).
13. McLellan, W. L., and Vogel, H. J., Proc. Nat. Acad. Sci. U. S. A., 67, 1703 (1970).
14. Udaka, S., Nature, 228, 336 (1970).
15. Blundell, M. R., and Wild, D. G., Biochem. J., 121, 391 (1971).
16. Faanes, R., and Rogers, P., J. Bacteriol., 96, 409 (1968).