First Enzyme of Histidine Biosynthesis and Repression Control of Histidyl-Transfer Ribonucleic Acid Synthetase of Salmonella typhimurium

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Received for publication 31 May 1974

The regulation of formation of histidyl-transfer ribonucleic acid (tRNA) synthetase was examined in strains of Salmonella typhimurium. When the first of the histidine-forming enzymes was wild type, the presence of 2-thiazolealanine in the growth medium prevented repression of histidyl-tRNA synthetase formation elicited by the addition of 1, 2, 4-triazole-3-alanine to these cultures. Conversely, thiazolealanine had no effect on repression of histidyl-tRNA synthetase formation by triazolealanine in hisG mutant strains. These data suggest a relationship between the control of histidyl-tRNA synthetase formation and the functional state of the histidine operon.

Recent investigations suggested that histidyl-transfer ribonucleic acid (tRNA) synthetase (E.C. 6.11.21 L-histidine tRNA ligase [adenosine 5'-monophosphate]) is regulated by a repression-like mechanism involving the cellular component histidyl tRNA (8). The results of previous studies indicate that histidyl tRNA (1), histidyl-tRNA synthetase (3), and the first enzyme of the histidine biosynthetic pathway (4, 5) are involved in the repression process of the histidine operon. In addition, a presumed commonality between the repression control of histidine pathway-specific enzymes and histidyl-tRNA synthetase has been suggested (8).

Specifically, for the histidine operon, Kovach et al. (5) demonstrated that if the hisG product activity is inhibited by the histidine feedback analogue (9), 2-thiazolealanine (TA), the repression pattern of the histidine pathway-specific enzymes is altered. In view of the apparent similarity in the control of synthesis of these two classes of histidine-specific enzymes, we conducted studies to determine whether the functional state of the first histidine biosynthetic enzyme (the hisG product) was related to the rate of histidyl-tRNA synthetase formation, as observed for control expression of the histidine operon. To examine the role of the hisG product in the regulation of histidyl-tRNA synthetase formation, the following histidine analogues were employed: (i) TA; which feedback inhibits the first enzyme of histidine biosynthesis, and (ii) triazolealanine (TRA) which mimics the addition of exogenous L-histidine in eliciting an excess signal for histidine biosynthesis and histidyl-tRNA synthetase formation. A preliminary report of some of these results has appeared (W. G. Coleman and L. S. Williams, Abstr. Annu. Meet. Amer. Soc. Microbiol., 1974, p. 192), and some of the studies reported are from a thesis submitted by W. G. C. to Purdue University in partial fulfillment of the requirement for the degree of Ph.D.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The organisms used in this study were LT-2 (wild type), SPII (ara-9 ilvC401 metE338 strA149, his-2179), the nonpolar, histidine auxotroph hisG52, the polar nonsense mutant hisG428, and the histidine auxotroph SB2800 (his 2321), a promoter mutant. These strains were obtained from P. Hartman and M. Levinthal. Isogenic pairs of hisG428 and hisG52 were made by transductions with P22 propagated on LT-2 by the method of Roth (10). Cells were routinely grown at 37 C in minimal salt medium with 0.4% glucose (11), and supplemented when necessary with 100 µg of the required amino acid(s) per ml (i.e., L-histidine, DL-methionine, L-isoleucine, and DL-valine). Growth was measured by an increase in optical density at 420 nm with a 1-cm light path in a Zeiss PMQ II spectrophotometer.

Preparation of crude extracts and enzyme assays. The preparation of crude extracts and the determination of histidyl- and leucyl-tRNA synthetases activities by the 14C-labeled amino acid attachment assay were as described by Chrisepeal et al. (2), with the exception that incubation time was 5 min. Protein was determined by the method of Lowry et al. (7).

Source of chemicals. 2-Thiazolyl-DL-alanine was

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obtained from Cyclo Chemical Co. 1,2,4-Triazole-3- 
alanine and tRNA (Escherichia coli K-12) were 
obtained from General Biochemicals. [U-14C]amino 
acids were obtained from New England Nuclear. All 
chemicals were reagent grade.

RESULTS

Effect of TA on the repression of histidyl-
tRNA synthetase formation in strain SPII. 
The repressibility of histidyl-tRNA synthetase 
formation was examined in strain SPIII which 
has a wild-type hisG product (Fig. 1). It was 
observed that, when the G enzyme activity was 
inhibited by TA in this strain, the addition of 
TRA did not lead to the repression of the hisS 
product (for procedural details see legend to 
Fig. 1). In the control culture, the addition of 
TRA resulted in the repression of histidyl-tRNA 
synthetase formation.

Effect of TA on the repression of histidyl-
tRNA synthetase formation in isogenic hisG 
and hisG+ strains. Isogenic pairs were 
constructed by crossing hisG mutants with phage 
prepared on hisG+ strains. These strains were 
examined to determine the repressibility of 

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\text{leucyl-tRNA synthetase; } \circ \text{, leucyl-tRNA synthetase. For proce-} 
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\text{dures see Fig. 1.} 
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\begin{align*}
\text{FIG. 1. Effect of TA on the repressibility of} \\
\text{histidyl-tRNA synthetase by TRA in strain SPIII. The} \\
\text{cells were initially grown in L-histidine unrestricted} \\
\text{medium (i.e., minimal glucose supplemented with} \\
\text{L-histidine, 100 } \mu \text{g/ml), harvested, washed twice with} \\
\text{minimal medium, and transferred to duplicate flasks,} \\
\text{one containing minimal medium and the other sup-} \\
\text{plemented with 0.1 mM TA. At the time indicated by} \\
\text{the arrow, TRA was added to a final concentration of} \\
\text{0.02 mM to both cultures. Histidyl-tRNA synthetase} \\
\text{activity was determined for the minimal culture (A) } \\
\text{and the TA-supplemented culture (O). In addition,} \\
\text{the activity of leucyl-tRNA synthetase was deter-} \\
\text{mined in minimal (} \circ \text{) and TA-supplemented cultures} \\
\text{(A). Results were plotted as enzyme units per millil-} \\
\text{iter of culture as a function of total protein per} \\
\text{milliliter of culture.} 
\end{align*}
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\text{FIG. 2. Effect of TA on the repressibility of histi-} \\
yl-tRNA synthetase by TRA in a hisS missense mutant, 
\text{hisG52. Minimal culture symbols: O, histi-} \\
dyl-tRNA synthetase; \circ , leucyl-tRNA synthetase. 
\text{TA-supplemented culture symbols: A, histidyl-tRNA} \\
\text{synthetase; A, leucyl-tRNA synthetase. For proce-} 
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\text{dures see Fig. 1.} 
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\begin{align*}
\text{FIG. 3. Effect of TA on the repressibility of histi-} \\
yl-tRNA synthetase in the presence of TA. The first isogenic pair examined was a hisG 
\text{missense mutant hisG52 and the hisG+ trans-} \\
ductant (Fig. 2). There was an apparent ab-} \\
\text{sence of an effect of TA on the repression of histidyl-tRNA synthetase by TRA in the hisG} \\
\text{mutant (hisG52). In contrast (Fig. 3), there was a lack of repression of histidyl-tRNA synthetase} \\
\text{formation in the hisG52+ strain (isogenic with strain hisG52) by TRA in the TA-containing} \\
\text{culture. Figure 4 shows the effect of TA on} 
\end{align*}
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histidyl-tRNA synthetase in a hisG nonsense (ochre) mutant (hisG428), and Fig. 5 shows the effect of TA on this enzyme in a hisG+ transductant of this mutant. It can be seen that TA has no effect on the repressibility of the synthetase in the absence of an active G enzyme, but prevents repression when the hisG product activity is genetically restored by transduction.

**Effect of TA on the repressibility of histidyl-tRNA synthetase in a histidine promoter strain.** Figure 6 shows the effect of TA on the hisS product in strain SB2800, a promoter mutant in which the histidine genes are intact, but in which none of the pathway-specific enzymes are formed. It is obvious that the addition of TRA to the TA-supplemented or minimal culture leads to an apparent repression of the hisS product.

**Effect of TA on leucyl-tRNA synthetase formation and growth.** As an external control, the leucyl-tRNA synthetase activity was determined. It was found (Fig. 1–4) that the rate of formation of this enzyme was not altered by the presence of TA or the addition of TRA. This suggests that the observed effect of TA on the repressibility of histidyl-tRNA synthetase by TRA is specific for this enzyme.

It should be noted that TA caused a histidine limitation in cells with a wild-type hisG product activity. This growth-inhibiting effect of TA was overcome by the addition of TRA. Furthermore, TRA stimulated growth of all the auxotrophic strains of *Salmonella typhimurium* used in this study and as reported by Levin and Hartman (6).

**DISCUSSION**

The results presented above clearly indicate a role of the first enzyme of histidine biosynthesis in the regulation of histidyl-tRNA synthetase formation. Previous studies (8, 12) indicated a role of the cognate amino acid and histidyl-tRNA in the repression control of histidyl-tRNA synthetase formation. These reports indicated a similarity between control of expression of histidine biosynthetic enzymes and histidyl-tRNA synthetase formation. As indicated above, we suggest still another biochemical unit common to the control of histidine biosynthesis and histidyl-tRNA synthetase. The results show that, when the first of the histidine-forming enzymes (hisG product) is wild type, the presence of TA in the growth medium prevents repression of histidyl-tRNA synthetase formation elicited by the addition of TRA to these cultures. Conversely, TA has no effect on repression of histidyl-tRNA synthetase formation by TRA in hisG mutant strains. Although the mechanism is not understood, these data...
suggest a relationship between the control of histidyl-tRNA synthetase formation and the functional state of the histidine operon. These results also provide new confirmation of the results of Kovach et al. (5).

Williams and Neidhardt (12) observed that histidyl-tRNA synthetase formation is regulated by a repression process in a parallel, but noncoordinate manner with that of the histidine biosynthetic enzymes. In addition, we observed that this synthetase is repressible by TRA in the presence of TA in hisG mutants. Perhaps these results are suggestive of a fine control mechanism, involving the G enzyme, which mediates a delicate balance between histidine biosynthesis and histidyl-tRNA synthetase formation.

Lastly, it should be emphasized that these results could perhaps be related to the internal pool of TA in normal and hisG mutant strains.

Since there is no evidence to our knowledge that TA is converted by the G enzyme to another compound or interacts with the synthetase, this latter question will be addressed in subsequent studies.

**ACKNOWLEDGMENTS**

This investigation was supported by a Public Health Service research grant GM 18905-02 from the National Institute of General Sciences. W. G. Coleman, Jr. was supported by Predoctoral Fellowship from the Southern Fellowship Fund. L. S. Williams is a Public Health Service Career Development awardee (K4-GM-32-981-02) from the National Institute of General Medical Sciences.

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