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Interference with the Feed-back Control of Histidine Biosynthesis*

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In bacteria, and probably in other organisms as well, the enzymes of amino acid, purine, and pyrimidine biosynthesis can function at higher rates and can be produced in greater amounts than are normally required. However, under the usual conditions of growth only a fraction of this potential is expressed. The degree of expression is controlled by the intracellular levels of end products in two ways: in many biosynthetic pathways the *action* of an early enzyme is inhibited by the end product (1–3); in addition, the formation of enzymes can be repressed by even slightly excessive amounts of the end product (4–6). These regulatory mechanisms permit bacteria to maintain a fine balance between the synthesis and the utilization of low molecular weight metabolites.

This balance can be disturbed by structural analogues which mimic the normal inhibitory effects of their corresponding metabolites on both enzyme action (7–9) and enzyme formation (10, 11). So far, only the former effect has been shown to be responsible for bacteriostasis. Thus, the bacteriostatic analogue 5-methyltryptophan acts by inhibiting, like tryptophan, an early step of tryptophan synthesis (9). Similarly (Diagram 1), the synthesis of an early precursor of histidine, Compound III, is inhibited not only by histidine (12) but also by the analogue 2-thiazolealanine (13). This mechanism of antimetabolite action has been described as false feed-back inhibition. In contrast, 6-methyltryptophan (11) and 8-azaguanine (10) are excellent mimics of their corresponding metabolites as repressors of enzyme formation but not as inhibitors of enzyme action. These analogues are relatively ineffective inhibitors of growth.

An antimetabolite which acts by interfering with a regulatory process should be useful in assessing the role of the process in the economy of the cell. The purpose of this paper is to describe the consequences of interference with a biological feed-back loop which can be achieved with the use of a false feed-back inhibitor of an essential biosynthetic reaction.

Materials and Methods

Chemicals—Dipotassium ATP and the magnesium salt of 5-phosphoribosyl-1-pyrophosphate (Pabst Laboratories), the barium salt of ribose-5-P, and glutathione (Schwarz Laboratories, Inc.), and dilithium acetyl-P (Cambridge Biochemicals Company) were commercial preparations. 2-Thiazolealanine, 2-pyridylalanine, and 1,2,4-triazolealanine were kindly provided by Dr. R. G. Jones of Eli Lilly and Company.

Analytic Methods—Histidine and histidinol were estimated by reaction with diazosulfanilic acid. Histidine was also estimated microbiologically by its ability to stimulate the growth of a histidine-requiring mutant, strain Hi B-12, of *Salmonella typhimurium*. The mutant was obtained from M. Demerec of the Long Island Biological Association.

Enzyme Assays—The synthesis of Compound III, and its conversion to 5-amino-1-ribosyl-4-imidazole-carboxamide 5′-phosphate and imidazoleglycerolphosphate, were estimated by previously described methods (12). Compound III for use as a substrate was partially purified by chromatography on a charcoal column.[1] This treatment completely removed adenine and adenine nucleotides. ACP[2] was present in all samples of Compound III. The best preparations contained 3% of this material.

Growth of Bacteria—All strains were grown with vigorous aeration at 37° in a mineral salts-glucose medium (9). Growth was estimated by measuring the turbidity of cultures in a Klett-Summerson colorimeter with a purple filter (No. 42) and is expressed as micrograms of dry weight per ml according to a previous calibration relating dry weight to turbidity.

RESULTS

Inhibition of Histidine Synthesis by 2-Thiazolealanine—Both histidine and 2-thiazolealanine inhibited the enzymatic synthesis of the histidine precursor, Compound III (13). In unfractionated extracts Compound III synthesis was 50% inhibited by 3.4×10^{-5} M histidine or by 1.2×10^{-3} M 2-thiazolealanine. Inhibition of the enzymatic reaction was independent of the concentration of the substrates, ribose-5-P and ATP. Because 5-phosphoribosyl-1-pyrophosphate had been shown to be required for the formation of Compound III (14), it too was tested and found to be incapable of competitively reversing the inhibitory effects of either histidine or 2-thiazolealanine.

If the site of action of 2-thiazolealanine is an early reaction in histidine synthesis such as the formation of Compound III, then cell growth and the ability of cells to synthesize imidazole-containing precursors of histidine should be equally sensitive to in-

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[1] H. S. Moyed, M. Civen, and B. Magasanik, unpublished observations.

[2] The abbreviations used are: ACP, 5-amino-1-ribosyl-4-imidazole-carboxamide 5′-phosphate; and IGP, imidazoleglycerolphosphate.

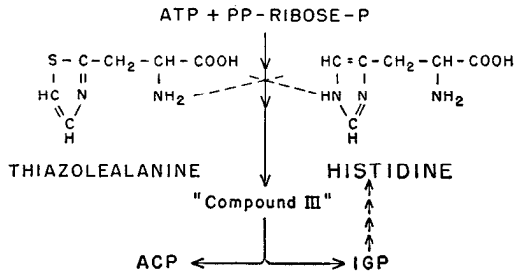


Diagram 1. Inhibition of the synthesis of an early precursor of histidine. Compound III is a partially characterized intermediate in the synthesis of the imidazole ring of histidine.

hibition by the analogue. In order to test this proposition histidine-requiring mutants of *Escherichia coli* strain W were isolated and examined for their ability to excrete the imidazole-containing precursors of the amino acid. Resting cell suspensions of one of these mutants, *E. coli* H-4, when incubated in a mineral salts-glucose medium, was found to excrete relatively large amounts of an imidazole which was provisionally identified as histidinol by chromatography in two solvent systems (15). Excretion of histidinol by the mutant and growth of the parent strain were equally sensitive to inhibition by 2-thiazolealanine (Fig. 1).

Antagonism of Bacteriostatic Effect of 2-Thiazolealanine by Histidine—The conclusion that 2-thiazolealanine functions by inhibiting histidine biosynthesis requires that histidine antagonize the bacteriostatic action of the analogue noncompetitively, provided the two compounds do not compete with each other for entry into the cell. Such a noncompetitive relationship between histidine and 2-thiazolealanine has been demonstrated. As shown in Table I the amount of histidine required for growth in the presence of 2-thiazolealanine was not appreciably affected by changes in the amount of this analogue.

2-Thiazolealanine-resistant Mutants—The probability that 2-thiazolealanine acts by mimicking the feed-back effect of histidine on the synthesis of Compound III suggested that mutants resistant to the analogue might produce a Compound III synthetase with altered sensitivity to feed-back inhibition. Such resistant organisms were selected by inoculating approximately 10^8 cells of *E. coli* strain W on minimal medium containing 10^{-3} M 2-thiazolealanine. This concentration of the analogue delayed the development of visible colonies by the sensitive parent strain for 5 or 6 hours. Several resistant mutants developed visible colonies without delay. The effects of 2-thiazolealanine on the growth rates of the two mutants and the parent strain were compared (Fig. 2). The growth rate of parent strain W was reduced 50% by 1×10^{-4} M 2-thiazolealanine, whereas 5 times as much of the analogue had no effect on strain TA-R_1 and little effect on strain TA-R_2.

Extracts of these mutants exhibited the anticipated alterations in sensitivity to the inhibitory effect of 2-thiazolealanine on the enzymatic synthesis of Compound III. As seen in Table II, the Compound III-synthesizing system of strain TA-R_1 was insensitive to the analogue, whereas the enzyme system of strain TA-R_2 was one-fourth as sensitive as that of the wild type organism. Table III shows essentially the same changes in the sensitivity of the enzyme system to its normal feed-back inhibitor, histidine.

Strain TA-R_1, unlike either strain W or strain TA-R_2, excreted considerable amounts of a compound which had the same electrophoretic mobility as histidine and, like histidine, produced a red chromatophore with diazosulfanilic acid and supported the growth of a histidine-requiring mutant of *S. typhimurium*, strain Hi B-12 (Table IV).

The fate of added histidine was compared in cultures of strain W and strain TA-R_1 (Fig. 3). The histidine content of both cultures remained unchanged during the first hour of the experiment. During exponential growth strain W rapidly removed histidine from the medium. In contrast, strain TA-R_1 not only failed to remove histidine, but actually excreted the amino acid during exponential growth. The sudden increase in the rate of histidine excretion by strain TA-R_1 at approximately 150 minutes coincided with the beginning of the decelerating phase of growth.

Strain TA-R_1 is obviously unable to adequately regulate its biosynthesis of histidine: it produces more histidine than it needs, and is unable to utilize exogenous histidine preferentially. Nevertheless, this mutant apparently retains its sensitivity to the repressive effect of histidine on the formation of enzymes necessary for histidine synthesis. The capacity of extracts of strains W and TA-R_1 to synthesize Compound III and also to convert it to ACP and imidazoleglycerolphosphate, the next step in histidine biosynthesis, is shown in Table V. Cells of strain W when grown in media containing 15 μg per ml of histidine had only about half the normal capacity for both the synthesis of Compound III and its conversion to ACP and imidazoleglycerolphosphate. The enzyme levels of the parent organism, strain W, could not be repressed below these values by additional histidine in the medium. The levels of the enzymes in strain TA-R_1 grown in minimal medium were nearly equal to the max-

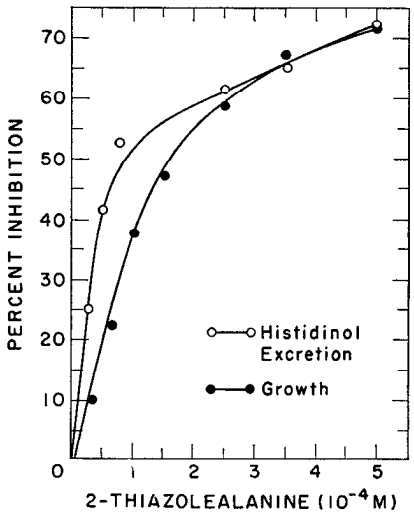


FIG. 1. Inhibition of growth and of histidinol excretion by 2-thiazolealanine. The growth rate of *E. coli* strain W in a mineral salts-glucose medium in the absence of the inhibitor was 1.4 generations per hour. Inhibition of growth is expressed as (1.4 − growth rate with inhibitor/1.4) × 100.

For measurement of histidinol excretion, cells of strain H-4 were suspended in a mineral salts-glucose medium (1 mg of cells per ml of medium) with the indicated amount of inhibitor and incubated at 37° with vigorous aeration. Samples were removed at 1 and 2 hours, and after the removal of cells by centrifugation the histidinol content of the medium was estimated by reaction with diazosulfanilic acid. Histidinol was excreted by *E. coli* strain H-4, a histidine auxotroph, at the rate of 5 μg per hour per mg of cells in the absence of the inhibitor. 2-Thiazolealanine itself did not form a colored compound with this reagent.

imally repressed levels of strain W, and the addition of histidine had no effect.

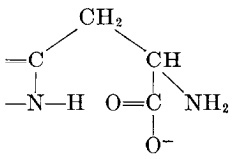
These results could indicate that strain TA-R_1, having lost sensitivity to the feed-back effect of histidine, is also insensitive to its repressive effect. However, a many-fold increase in the levels of these activities is known to occur when the histidine supply of auxotrophs is limited. A similar increase should occur in strain TA-R_1 if the organism had lost sensitivity to repression by histidine. A more plausible explanation is that strain TA-R_1 has retained the repressibility of its parent, and that the overproduction of histidine which results from the absence of feed-back inhibition causes maximal repression; hence, an external supply of histidine would have no additional effect.

Nonheritable (Phenotypic) Resistance to 2-Thiazolealanine—It was observed that the addition of 2-thiazolealanine to cultures in exponential growth caused immediate bacteriostasis; however, after a relatively short interval, exponential growth was resumed at a rate which was somewhat lower than that of the control (Fig. 4). Cells which have "recovered" in this manner from the effects of 2-thiazolealanine did not show a lag in their growth when re-exposed to the analogue, but their resistance was lost by cultivation in the absence of the analogue (16). This development of phenotypic resistance was accompanied by a 20-fold increase in the level of the sensitive enzyme system, Compound III synthetase; maintenance of the elevated enzyme level, like resistance, was dependent on cultivation in the presence of the analogue (16).

Within narrow limits the increase in Compound III synthetase activity was proportional to the concentration of 2-thiazolealanine. It is of some interest to note that even small amounts of 2-thiazolealanine, which did not affect growth, caused a marked stimulation of Compound III synthetase (Table VI). In addition, 2-thiazolealanine caused a parallel increase in the next enzyme in the sequence, which converts Compound III to ACP and imidazoleglycerolphosphate (Fig. 5).

The ability of 2-thiazolealanine to "induce" the early enzymes of histidine biosynthesis is a direct function of its ability to inhibit the action of Compound III synthetase. Strain TA-R_2 was one-fourth as sensitive as strain W to "induction" of the early enzymes of histidine synthesis by 2-thiazolealanine, whereas strain TA-R_1 was totally unaffected by the analogue in the same concentration range (Fig. 6). The degree of sensitivity to "induction" by 2-thiazolealanine is the same as to its inhibition of Compound III synthesis already shown in Table II.

Other Inhibitors of Compound III Synthesis—It was found that 2-pyridylalanine and 1,2,4-triazolealanine were also inhibitors of Compound III synthesis, whereas neither histamine nor urocanic acid was active. The active compounds have in common an alanine side chain attached to a carbon atom in the heterocyclic ring which is adjacent to a nitrogen atom. The essential structure would appear to be:



This conclusion is supported by the observation that the simplest compound possessing this structure, α,γ-diaminobutyric acid, was active (Fig. 7). Compared to the heterocyclic compounds, α,γ-diaminobutyric acid was a weak inhibitor; however, like 2-thiazolealanine, it appeared to be selective in its action on Compound III synthesis inasmuch as growth in its presence (2×10^{-3} M) caused a 3-fold stimulation in the formation of Compound III synthetase. Both amino groups appeared to be involved in the inhibitory effect as neither α-aminobutyrate, γ-aminobutyrate, nor a combination of the two inhibited Compound III synthesis.

Table I

Antagonism of bacteriostatic effect of 2-thiazolealanine by histidine

The inoculum was an exponentially growing culture of *E. coli* strain W. The initial bacterial density was 5 μg per ml. The cultures were incubated for 5 hours.

2-Thiazolealanine	Growth in presence of L-histidine (M × 10^{-5})				
	0	0.5	1.0	1.5	2.5
M × 10^{-4}	μg*/ml				
	206				
2.0	6	75	91	103	141
4.0	4	67	81	93	136
6.0	5	59	78	98	137

* Dry weight.

Although 2-pyridylalanine and 1,2,4-triazolealanine are both inhibitors of Compound III synthesis *in vitro*, this effect cannot be responsible for their bacteriostatic action. First, neither mutant selected for resistance to 2-thiazolealanine showed increased resistance to 2-pyridylalanine; and only one of the mutants, strain TA-R_1, which excretes histidine, was resistant to 1,2,4-triazolealanine. This pattern of resistance to bacteriostasis was made more interesting by the finding that the mutant Compound III-synthesizing systems with reduced sensitivity to 2-thiazolealanine showed parallel reductions in sensitivity to both 2-pyridylalanine and 1,2,4-triazolealanine.

The locus responsible for the bacteriostatic action of 2-pyridylalanine was revealed by the observations made here and previously in another laboratory (17) that this analogue was antagonized by phenylalanine but not by histidine. In contrast

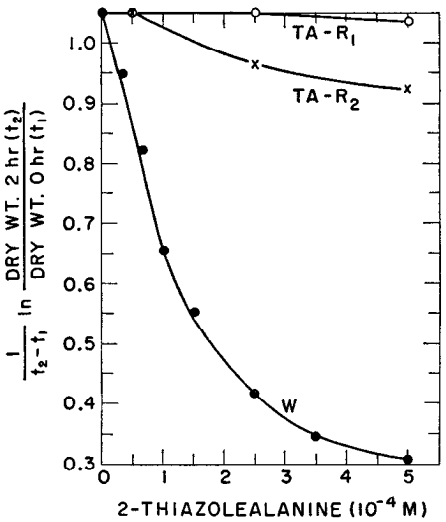


Fig. 2. The effect of 2-thiazolealanine on the growth of *E. coli* strain W and of resistant mutants, strains TA-R_1 and TA-R_2. Each culture contained 25 μg per ml of bacteria in the exponential phase of growth at the beginning of the experiment.

TABLE II

Inhibition of enzymatic synthesis of Compound III by 2-thiazolealanine in extracts of 3 strains of E. coli

2-Thiazolealanine	Compound III formation		
	Strain W	Strain TA-R_1	Strain TA-R_2
M	*mμmoles × hr⁻¹ × mg protein⁻¹*		
	120	46	82
1.00×10^{-3}	66		
1.25×10^{-3}	60		
1.50×10^{-3}	48		
2.00×10^{-3}	32		62
4.00×10^{-3}	20		40
5.00×10^{-3}		54	
6.00×10^{-3}			34
1.00×10^{-2}		50	
1.50×10^{-2}		54	

TABLE III

Inhibition of enzymatic synthesis of Compound III by histidine in extracts of 3 strains of E. coli

L-Histidine	Compound III formation		
	Strain W	Strain TA-R_1	Strain TA-R_2
M	*mμmoles × hr⁻¹ × mg protein⁻¹*		
	118	60	117
3.33×10^{-5}	81		110
5.00×10^{-5}	71		98
6.67×10^{-5}	49		98
1.67×10^{-4}			75
2.50×10^{-4}			50
3.33×10^{-4}		62	
6.67×10^{-4}		64	
1.00×10^{-3}		62	

1,2,4-triazolealanine was antagonized by histidine, but unlike 2-thiazolealanine, its relationship to histidine was competitive. In further contrast to 2-thiazolealanine, the formation of Compound III synthetase was inhibited rather than stimulated by 1,2,4-triazolealanine. Finally, inhibition of growth by the triazole was not immediate. Instead, shortly after the addition of the analogue the growth rate progressively decreased.

The effects of 1,2,4-triazolealanine on growth and on enzyme formation could indicate that the analogue acts either by mimicking the repressive effect of histidine on the formation of enzymes involved in histidine biosynthesis, or by getting incorporated into protein in place of histidine. The latter interpretation better fits the observation (Fig. 8) that growth in the presence of 1,2,4-triazolealanine reduced the specific activity not only of histidine-synthesizing enzymes but also of unrelated enzymes such as inosinic dehydrogenase and tryptophan synthetase. It should be noted that the levels of the three enzymatic activities shown in Fig. 8 were not equally affected by growth in the presence of the histidine analogue. Such results might be anticipated from the reasonable assumption that histidine does not have an equally important role in the catalytic function of each of the enzymes.

Discussion

The observation that several structural analogues of histidine can mimic the inhibitory effect of this amino acid on the enzymatic synthesis of one of its early precursors, Compound III, suggested a possible site for the action of the analogues. However, further examination of the effects of these analogues illustrated that the demonstration of an inhibitory effect on an isolated enzyme system is inadequate as sole evidence for the mechanism of bacteriostatic action. The inhibitory effect on Compound III synthesis by two of the three analogues used in this study, 2-pyridylalanine and 1,2,4-triazolealanine, proved fortuitous: the principal action of the former compound was as an antagonist of phenylalanine, whereas the latter compound appeared to act by getting incorporated into protein. On the other hand, the bacteriostatic action of a third analogue, 2-thia-

TABLE IV

Excretion of histidine by a 2-thiazolealanine-resistant mutant

All three strains of Table II were incubated until the bacterial density had increased from 40 to 350 μg per ml. Cells were then removed by centrifugation. The culture fluid of strain TA-R_1 was passed over a column of Dowex 50-H^+. The column was washed with water and then with 1 N NH_4OH. The NH_4OH wash was concentrated and subjected to paper electrophoresis, along with a known sample of histidine, in 0.04 M citrate, pH 3.2. The paper was developed by spraying with diazosulfanilic acid.

Method of analysis of filtrate	Histidine in culture fluid			Mobility
	Strain W	TA-R_1	TA-R_2	
	μg ml			*cm × hr⁻¹**
Reaction with diazosulfanilic acid	1	9	1	
Microbiological	0	7		
Paper electrophoresis at pH 3.2				
L-Histidine				0.68
Product extracted by TA-R_1				0.67

* 25 volts per cm.

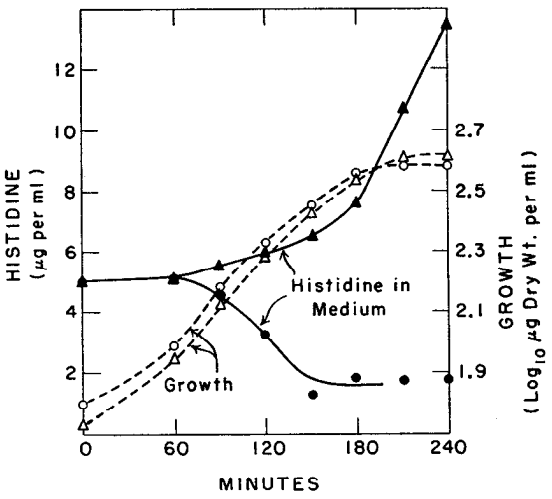


FIG. 3. Histidine utilization and excretion during growth by *E. coli* strain W and by a 2-thiazolealanine-resistant mutant, *E. coli* strain TA-R_1. Both organisms were grown in media supplemented with 5 μg per ml of histidine. The histidine content of the medium was estimated at the indicated intervals by its reaction with diazosulfanilic acid. Histidine in the medium: strain W, ●——●; strain TA-R_1, ▲——▲. Growth: strain W, ○ - - ○; strain TA-R_1, △ - - △.

TABLE V

Effect of growth in presence of histidine on formation of enzymes of histidine synthesis

Each culture was incubated for 16 hours. The yield of cells in each case was 0.6 g per liter.

Strain	Addition to growth media	Compound III synthesis	Conversion of Compound III to ACP
	mg histidine/l	*mμmoles* × *hr*$^{-1}$ × *mg protein*$^{-1}$	
W	0	125	144
W	40	60	80
TA-R_1	0	50	64
TA-R_1	40	50	60

zolealanine, depended principally on its ability to inhibit Compound III synthesis.

Evidence for such a site of action is based, in part, on the following results: (*a*) accumulation of histidinol, an obligatory precursor of histidine, by resting cells of a histidine auxotroph and growth of the wild type were equally sensitive to inhibition by 2-thiazolealanine; (*b*) the bacteriostatic action of 2-thiazolealanine was overcome noncompetitively by the addition of histidine to the medium; and (*c*) mutants selected for resistance to 2-thiazolealanine produced Compound III-synthesizing systems with decreased sensitivity to the analogue.

These enzymes showed a parallel decrease in sensitivity to the normal inhibitory effect of histidine. The partial loss of sensitivity of the enzyme system in one mutant had no other detectable physiological consequence than increased resistance to the analogue. In contrast, another mutant which produced a completely insensitive enzyme system had undergone a marked change in its physiology. Unlike the parent strain it produced twice as much histidine as it could use for protein synthesis, and was no longer able to utilize exogenous histidine preferentially.

The latter mutant retained the repressive effect of histidine on the formation of enzymes necessary for histidine biosynthesis, but nevertheless the organism was obviously unable to control its synthesis of histidine. Thus, in the present case it is shown that feed-back inhibition of an early enzyme is essential for regulation of end product synthesis, and that such regulation cannot be accomplished by adjustment of enzyme levels through repression. This inability of histidine to regulate its own synthe-

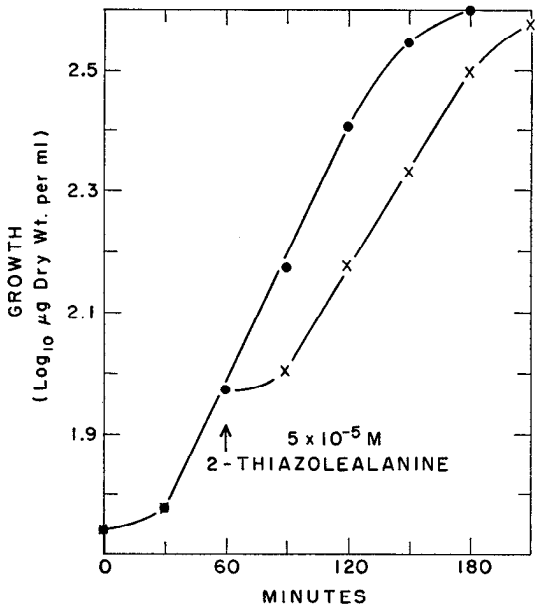


FIG. 4. Transitory inhibition of growth by 2-thiazolealanine. The analogue was added after 60 minutes to one of two parallel cultures of *E. coli* strain W.

TABLE VI

Induction by 2-thiazolealanine of resistance and of increased capacity for synthesis of Compound III

Each culture of *E. coli* strain W was incubated until the bacterial density had increased from 25 to 400 μg per ml. The cells were then collected by centrifugation and extracts were prepared by sonic oscillation. The growth rate constant is $\frac{1}{t_2 - t_1} \ln \frac{\text{dry weight at } t_2}{\text{dry weight at } t_1}$, and was determined for each culture during exponential growth after the lag phase was over.

2-Thiazolealanine	Compound III synthesis	Duration of lag phase	Growth rate constant
M × 10^{-5}	*mμmoles* × *hr*$^{-1}$ × *mg*		
	53	0′	1.0
1.0	179	0′	1.0
3.0	240	8′	0.8
5.0	640	22′	0.7
7.0	650	38′	0.6
10.0	795	75′	0.6

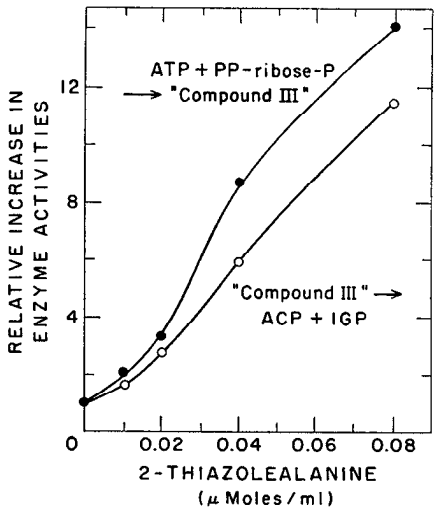


FIG. 5. The effect of 2-thiazolealanine on the formation of enzymes of histidine biosynthesis by *E. coli* strain W. The cultures were incubated until the cell mass had increased from 30 to 250 μg per ml. Cells from 500 ml of such cultures were centrifuged, washed twice with 0.03 M potassium phosphate buffer at pH 7.4, suspended in 12 ml of buffer, and subjected to sonic oscillation for 5 minutes. The extracts were clarified by centrifugation at 18,000 × *g* for 15 minutes. Protein was determined by the Biuret method with crystalline bovine serum albumin as the standard. The synthesis of Compound III and its conversion to ACP and IGP were estimated according to the method of Moyed and Magasanik (12). One mg of protein in the extract prepared from cells grown in the absence of 2-thiazolealanine synthesized 0.053 μmole of Compound III per hour and converted 0.042 μmole of Compound III to ACP per hour. Compound III synthesis was measured in the absence of an amino donor which prevented its conversion to ACP. The conversion of Compound III to ACP was estimated in the absence of ATP and 5-phosphoribosyl-1-pyrophosphate, the substrates for Compound III synthesis.

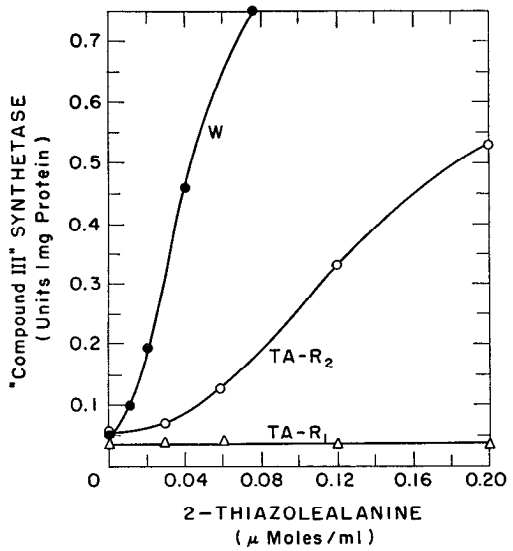


FIG. 6. The effect of growth in the presence of 2-thiazolealanine on the level of Compound III synthetase in *E. coli* strain W and in the 2-thiazolealanine-resistant strains, TA-R_1 and TA-R_2. See Fig. 5 for conditions of growth, preparation of extracts, and determination of Compound III synthesis. A unit is equal to the formation of 1 μmole of Compound III in an hour.

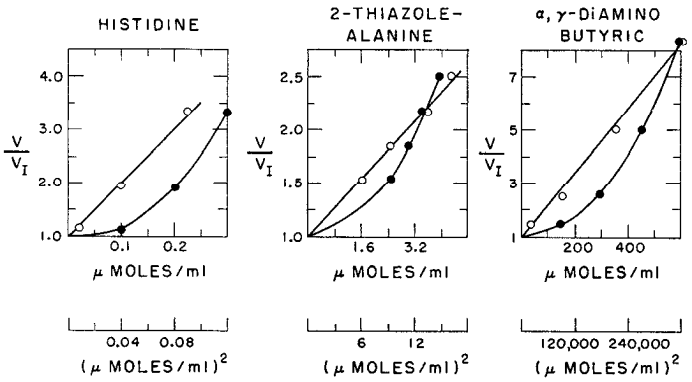


FIG. 7. Comparison of the effects of histidine, 2-thiazolealanine, and α,γ-diaminobutyric acid on the enzymatic synthesis of Compound III. The extract used in these experiments was prepared from cells of *E. coli* strain W grown in the presence of 0.077 μmole per ml of 2-thiazolealanine (see Fig. 5). Compound III synthesis was determined as described by Moyed and Magasanik (12) except that 5-phosphoribosyl-1-pyrophosphate was used in place of ribose-5-P. V, the rate of Compound III synthesis in the absence of inhibitor, was 0.027 μmole in 10 minutes with 0.05 ml of extract (5.7 mg of protein per ml). V_1 is the rate of reaction with the indicated amount of inhibitor. ●——●, μmoles per ml; ○——○, (μmoles per ml)².

sis by repression alone is due to the fact that maximal repression causes only a 50% decrease in the normal levels of the necessary enzymes. As synthetic enzymes in general are not susceptible to complete repression, the loss of sensitivity to feed-back inhibition, when it occurs, should cause overproduction of a metabolite. A possibly converse situation has been described in which the loss of repressibility of the enzymes of arginine biosynthesis did not result in overproduction of arginine, presumably because one of the enzymes was still sensitive to feed-back inhibition by the amino acid (18).

The finding that a mutant can produce an enzyme which has become completely insensitive to feed-back inhibition by histidine while remaining susceptible to the repressive effect of histidine on its formation argues that these processes are independent of each other. This dichotomy is reflected by the fact that 2-thiazolealanine can mimic the inhibitory effect of histidine on enzyme action but not its repressive effect on enzyme formation. As a result, bacteria are able to make a compensation which permits them to recover fairly rapidly from the bacteriostatic effect of 2-thiazolealanine. The analogue creates a histidine deficiency by inhibiting Compound III synthesis, thereby retarding growth. However, the deficiency also relieves repression of the formation of the enzymes necessary for histidine synthesis. The consequent increased level of the sensitive enzyme permits resumption of a nearly normal growth rate despite the presence of the inhibitor.

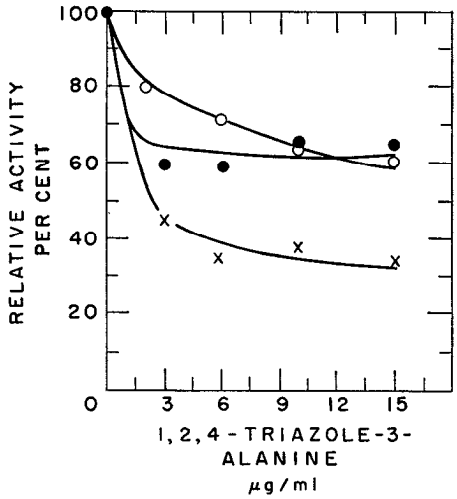


FIG. 8. The effect of 1,2,4-triazolealanine on the formation of several enzymes by *E. coli* strain W during growth. See Fig. 5 for conditions of growth and for preparation of extracts. The synthesis of Compound III, ●——●, was estimated according to the method of Moyed and Magasanik (12); inosinic dehydrogenase, ○——○, was assayed spectrophotometrically (19); and tryptophan synthetase, ×——×, was estimated by the method of Yanofsky (20).

The effect of 2-thiazolealanine is reminiscent of the response of an inducible enzyme to its inducer: greatly elevated levels of Compound III synthetase are produced by cells grown in a medium containing 2-thiazolealanine, but these soon return to normal values during subsequent growth unless the analogue is present. According to current theories of the action of inducers of enzyme formation, the role of the inducer is to antagonize an unknown but specific repressor of enzyme formation. The demonstrated mechanism of action of 2-thiazolealanine formally fits this model: it induces the formation of Compound III synthetase by antagonizing the formation of the repressor, histidine. A similar response of tryptophan synthetase to 3-methylanthranilic acid, an inhibitor of tryptophan biosynthesis, has been reported (12).

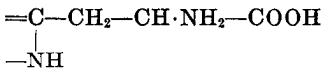
SUMMARY

Histidine is a feed-back inhibitor of the enzymatic synthesis of one of its early precursors, Compound III. A structural analogue of histidine, 2-thiazolealanine, also inhibits this synthesis. Evidence is presented that this inhibitory effect is responsible for bacteriostasis by the analogue. The acquisition of genotypic resistance to the analogue results from the develop-

ment of an enzyme system which is insensitive not only to 2-thiazolealanine but also to histidine itself. This mutant retains normal sensitivity to the repressive effect of histidine on the formation of enzymes necessary for histidine synthesis. However, repression by itself is not adequate for the regulation of histidine synthesis, and the mutant overproduces and excretes the amino acid during growth.

Bacteria can also develop phenotypic resistance to 2-thiazolealanine in the following manner: the analogue inhibits the synthesis of an early histidine precursor, thereby creating a histidine deficiency and reducing the growth rate; this deficiency relieves the repressive effect of histidine so that increased amounts of the histidine-synthesizing enzymes are formed, including the one which is sensitive to 2-thiazolealanine; growth can now be resumed despite the presence of the analogue. Both phenotypic resistance and the increased enzyme levels are lost during growth in the absence of the analogue.

A comparison of the ability of compounds to mimic histidine as a feed-back inhibitor suggests that the necessary configuration is



as in histidine, 2-thiazolealanine, 1,2,4-triazolealanine, 2-pyridylalanine, and the aliphatic compound α,γ-diaminobutyric acid.

Two analogues were found to inhibit Compound III synthesis, although one had its principal effect as a phenylalanine antagonist and the other as a replacement for histidine in protein synthesis.

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