Induced Phenotypic Resistance to an Antimetabolite

Abstract. Resistance to bacteriostasis by 2-thiazole alanine develops rapidly; however, such resistance is lost during growth in the absence of the analog. This induced resistance is accompanied by increased formation of an enzyme sensitive to 2-thiazole alanine. Maintenance of the elevated enzyme levels in growing cells, like resistance, requires the presence of the analog.

An antimetabolite, 2-thiazole alanine, has been shown to retard the growth of *Escherichia coli* W by mimicking the specific inhibitory effect of its corresponding metabolite, histidine, on the action of "compound III" synthetase, an early enzyme of histidine biosynthesis (*1*). Further investigation reveals that this false feedback inhibitor has an immediate but transitory bacteriostatic effect; after a brief period, growth is resumed at an exponential rate somewhat lower than usual. Both the duration of bacteriostasis and the extent of the reduction in the rate of the subsequent growth increase with increasing concentration of the analog.

It is extremely unlikely that the temporary nature of the bacteriostasis is due to selection of resistant mutants, for resumption of growth occurs after relatively brief periods (up to 1 hour). Destruction of the analog also appeared to be an unlikely explanation, for the bacteriostatic action of 2-thiazole alanine lasts for a much shorter period than its inhibitory effect on the excretion of histidinol by nongrowing cells of a mutant which cannot convert this intermediate to histidine. This conclusion is also supported by the results shown in Fig. 1. Cells which have recovered from the initial inhibition by 2-thiazole alanine grow without a lag when transferred to fresh media containing the inhibitor; however, if the cells are allowed to grow sufficiently in normal media before re-exposure to the antimetabolite, the initial sensitivity of the strain is again observed. 2-Thiazole alanine thus appears to be an inducer of resistance to its bacteriostatic action.

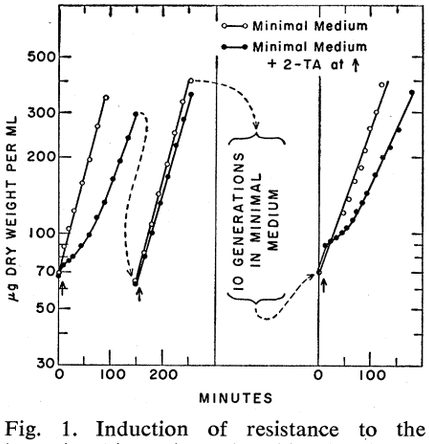

Fig. 1. Induction of resistance to the bacteriostatic action of 2-thiazole alanine (2-TA). The inoculum was an exponentially growing culture of *Escherichia coli* W. The minimal medium contained, per liter: 18.9 gm of $Na_2HPO_4 \cdot 7H_2O$, 6.3 gm of KH_2PO_4, 0.2 gm of $MgSO_4 \cdot 7H_2O$, 0.01 gm of $CaCl_2$, 2 gm of $(NH_4)_2SO_4$, 2 gm of glucose, and 2 mg of 2-thiazole alanine as indicated. The cultures were aerated by rotary shaking at 37°C. The arrows indicate the transfers made during the experiment. Growth was measured as change in optical density and is expressed as micrograms of dry weight of bacteria per milliliter from a previous calibration.

An explanation for this apparent paradox was suggested by assuming that both the inhibition of growth and the subsequent induction of resistance are consequences of a single activity of 2-thiazole alanine: inhibition of the synthesis of the histidine precursor, "compound III." The resulting decrease in the intracellular supply of histidine would decrease the growth rate, but it would also relieve the repressive effect of histidine on the formation of "compound III" synthetase (*2*) so that an increased amount of this enzyme system, which is sensitive to 2-thiazole alanine, might be formed (*3*). In this manner "compound III" synthesis could be resumed despite the presence of 2-thiazole alanine.

This explanation is supported by observations on the cellular content of "compound III" synthetase. Growth in a medium containing a small amount of the antimetabolite caused a threefold increase in the specific activity of the enzyme in extracts of the cells. Maintenance of the elevated activity in growing cells was dependent on the continued presence of 2-thiazole alanine (Fig. 2). Increasing the levels of the analog in growing cultures causes parallel increases in the level of the enzyme within the cell, up to 20 times the normal.

This response to 2-thiazole alanine bears a formal resemblance to induced enzyme formation. The analog permits induction of "compound III" synthetase by inhibiting the synthesis of histidine, which would normally prevent extensive formation of this enzyme system. While these observations may not be relevant to the action of the usual inducers of enzyme formation, it is interesting to note the recent evidence that at least one inducer functions by antagonizing an unknown but specific represser of enzyme formation (*4*).

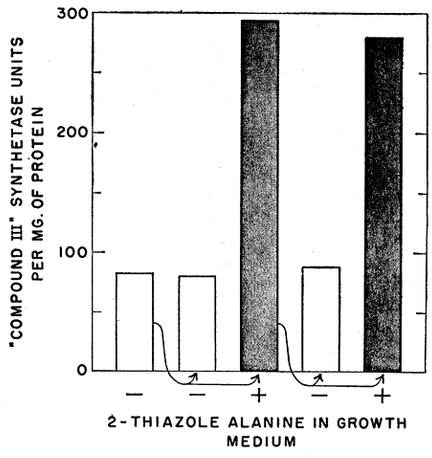

Fig. 2. Induction of "compound III" synthetase formation by 2-thiazole alanine. A unit of "compound III" synthetase activity corresponds to the formation of 0.4 μmole of the compound per hour. Enzymatic activity was determined by the method of Moyed and Magasanik (*2*). The cells were grown in the medium described in Fig. 1 and were harvested while still in the exponential phase of growth. Extracts were prepared by sonic oscillation. The original inoculum was a 16-hour culture of *Escherichia coli* W in minimal medium. The subsequent transfers are indicated by arrows. After each transfer the cultures were incubated until a 15-fold increase in cell mass had occurred.

Similar responses by bacterial and mammalian cells to other enzyme inhibitors could be important factors in the failure of many theoretically useful compounds to inhibit cell growth effectively at low doses (*5*).

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References and Notes

1. H. S. Moyed and M. Friedman, *Science* **129**, 968 (1959); *Federation Proc.* **18**, 290 (1959).
2. H. S. Moyed and B. Magasanik, *J. Biol. Chem.* **235**, 149 (1960).
3. Such a stimulation of the production of "compound III" synthetase was observed when the intracellular level of histidine was depleted by another technique in which a histidine auxotroph is forced to obtain the amino acid at a low rate from β-alanylhistidine. The use of a combined form of histidine for this purpose was suggested → B. N. Ames and B. Garry, *Proc. Natl. Acad. Sci. U.S.* **45**, 1453 (1959).
4. A. B. Pardee, F. Jacob, J. Monod, *J. Molecular Biol.* **1**, 165 (1959).
5. This work was supported by U.S. Public Health Service grant RG-6059. The 2-thiazole alanine was donated by R. G. Jones of the Lilly Research Laboratories.

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