# CONSTITUTIVE DEGRADATION OF PUTRESCINE IN A PSEUDOMONAS SPECIES AND ITS POSSIBLE PHYSIOLOGICAL SIGNIFICANCE

Ruth Michaels and T. T. Tchen
Department of Chemistry
Wayne State University
Detroit, Michigan 48202

Received December 23, 1970

Summary It is well known that the polyamines are normally not "degraded" by E. coli but that they can be "inactivated" by acetylation. This paper documents the fact that a Pseudomonas species, which does not acetylate putrescine, has a constitutive degradative pathway for putrescine. A hypothesis is presented that these two modes of inactivation of polyamines may represent an essential regulatory mechanism, aside from the classical repression and feedback inhibition, on the intracellular level of these compounds. It is suggested that such regulation, involving degradation or other means of inactivation, may be a general one applicable to synthetic end products which serve a regulatory function.

## Introduction

We wish to document the observation that a Pseudomonas species which does not inactivate putrescine by acetylation has a constitutive pathway of putrescine degradation. This raises the possibility that the activity of inactivating systems (acetylation or degradation), aside from regulatory mechanisms on synthetic activities, may serve as an essential component in the regulation of the intracellular polyamine level in bacteria.

## Materials and Methods

Cells are grown to early log phase in Casaminoacids medium as previously described.[1] Trace amounts of labeled putrescine 1,4-$^{14}$C (1 $\mu$ci, 0.04 $\mu$moles per 100/ml of culture) were added to the culture and incubation continued for 25 minutes at which time approximately half of the label was taken up by the cells. The labeled cells were collected by centrifugation (3000 xg, 10 minutes, room temperature) and re-incubated in fresh unlabeled medium (same volume as original culture). After 60 minutes of incubation, the cells were collected by

millipore filtration. Aliquot of the filtrate were taken for $^{14}C$ determination in a liquid scintillation counter (counting medium: 250 gms naphthalene, 15 gms. PPO (Packard), 600 ml ethanol and 1 l. each of toluene and dioxane). The remainder of the filtrate was acidified with trichloroacetic acid (final concentration 5%) to remove $CO_2$. Trichloroacetic acid was removed by ether extraction ( 3 times with no loss of $^{14}C$) and the aqueous solution, after addition of a small volume of concentrated HCl, was evaporated to dryness. After re-dissolution in a small volume of water, an aliquot corresponding to 3 ml of the original culture medium was applied to a column of Dowex 50 W-x-4 (200 mesh, 1.2 cm.x 15 cm.).

After washing with 20 ml of water which eluted no radioactivity, amino acids including 4-aminobutyrate were eluted with 20 ml of 3N HCl and then the diamines eluted with 20 ml of 6N HCl. Aliquots of these eluates were counted for radioactivity. The remainder of the 6N HCl was evaporated to dryness, re-dissolved, mixed with cold carrier putrescine and subjected to thin layer chromatography on silica gel (developed by methanol-concentrated ammonias 3:1 v/v). The putrescine band was visualized by iodine vapor, scraped off the plate, eluted with methanolic HCl (4:1 v/v) and counted.

The cells, collected on millipore filter, were extracted twice (overnight) with 5% trichloroacetic acid. The combined extract was extracted three times with ether to remove the trichloroacetic acid. The aqueous solution was further acidified with a few drops of concentrated HCl and dried in vacuo over KOH. The residue was dissolved in a small volume of water, mixed with carrier putrescine and hydroxyputrescine and chromatographed on silica gel plates. The bands of putrescine and hydroxyputrescine were again visualized with iodine vapor, scraped off the plates and counted. The trichloroacetic insoluble material was collected on millipore filter, dried and counted (non-aqueous counting medium: 9 g PPO, 900 mg dimethyl POPOP (packard), 3 l. toluene).

## Results

The distribution of $^{14}C$ in the various fractions after 60 minutes of incubation is shown in Table I. It is clear that even in a complex medium like Casaminoacids there is extensive degradation of the intracellular putrescine. Assuming an intracellular putrescine content of 10 mM[2], it is calculated that the rate of putrescine degradation is approximately 0.07 μmole/min/gm wet weight of cells.

TABLE I

| Fraction and Subfraction | | $^{14}C$ as % of Original intracellular $^{14}C$-putrescine |
|---|---|---|
| Medium | Total | 42 |
| | Acid volatile | 30 |
| | Non acid volatile | 12 |
| | 3N HCl eluate | 1 |
| | 6N HCl eluate | 11 |
| | Putrescine | 9 |
| Cells | Trichloroacetic soluble | |
| | Putrescine | 39 |
| | Hydroxyputrescine | 17 |
| | Other compounds | <2 |
| | Trichloroacetic insoluble | 3 |

## Discussion

We have been studying the metabolism of polyamines in this Pseudomonas species. This organism is unique in that it contains hydroxyputrescine aside from putrescine but has neither the triamine, spermidine, nor any acetylated polyamines.[2, 3] Studies on two of the enzymes (4-aminobutanal and succinic semialdehyde dehydrogenases) usually involved in putrescine degradation showed that these enzymes are present in cells grown in all media tested with the exception that 4-aminobutanal dehydrogenase is undetectable in cells grown in the complex medium of Casaminoacids.[1]

The results presented here show that putrescine degradation takes place in the Casaminoacids medium as well. This apparent discrepancy between the earlie enzymatic studies (non-detectable level of 4-aminobutanal dehydrogenase) and the present turnover studies is probably due to the fact that the observed rate of putrescine degradation needs very low levels of this dehydrogenase, which would be "non-detectable" under conditions of the enzymatic studies.

The finding that putrescine degradation is constitutive in this organism can be explained by either of two hypotheses. First, this happens to be an unusual mutant where this degradative pathway is constitutively de-repressed by chance and does not have any special significance. Alternatively, one may theorize that this degradative pathway serves an important, perhaps regulatory,function. We favor the second hypothesis for the reason outlined below.

In E. coli, it has been known for some time that although there is usually "no degradation" of the polyamines, the polyamines can be inactivated by acetylation, a process which is triggered by chilling or by the presence of excess spermidine in the medium.[4] Since acetylated polyamines are present in other bacteria, it is possible that this inactivating mechanism is present also in these organisms and that it serves as part of the mechanism for the regulation of the level of polyamines. The Pseudomonas species under study here does not have acetylputrescine and presumably does not have an acetylation mechanism for polyamines.[2] Instead, we find that this organism contains a constitutive degradative pathway for putrescine. It is our belief that this degradative pathway, similar to the acetylating system in E. coli, serves to regulate the intracellular level of polyamines.

Since the current concepts on regulation of anabolic pathways in bacteria emphasize the dual regulation by repression and feedback inhibition, with the tacit assumption that degradation of these products of synthetic pathways are usually repressed, it is pertinent to ask whether there should be mechanism(s) for inactivation and/or degradation of such products in order to regulate the levels of these compounds. It should be noted that the current concepts on the regulation of synthetic pathways are based mostly on the synthesis of amino acids and nucleotides that serve as precursors for macromolecules. However, if the product of a synthetic pathway were to serve a regulatory function, it would be logical to have a dual mechanism of regulation of its level, one by synthesis and the other by degradation. Such mechanisms are of course well known in higher organisms such as the synthesis and degradation of hormones and of

cAMP. Although procaryotic cells do not have hormones, the important role of cAMP in the regulation of certain genetic expression in bacteria has been demonstrated recently. In this case, the level of cAMP appears to be regulated by synthesis, excretion and perhaps degradation. (See reference 5).

In the case of the bacterial polyamines, there are evidence that they are involved in the regulation of RNA and/or protein synthesis.[6] It seems, therefore, reasonable to assume that there should be regulation of polyamine content by a combination of synthesis and removal. In some bacteria, this removal is achieved by acetylation. In the Pseudomonas species discussed here, degradation appears to be the principal removal mechanism. Excretion into the medium also contributes to the removal of intracellular putrescine but is quantitatively less important. Conversion to hydroxyputrescine serves an unknown function but does not result in the removal of polyamines as it only converts one diamine to another.

Although there are probably many low molecular weight compounds (such as fructose-1, 6-diphosphate) in bacteria which could serve a regulatory function, most of these are also normal intermediates of some major metabolic pathway and mechanisms for their removal are normally present in the cells. However, if one should encounter low molecular weight regulatory molecules which, like cAMP and the polyamines, are not intermediates in normal metabolic pathways, one should find constitutive mechanisms for their removal, including the possibility of constitutive degradative enzymes.

## Acknowledgement

This work was supported by grants AM5384 and AM13724 from the USPHS. The authors wish to thank Drs. T. Kimura and H. McAllister for helpful discussions.

## References

1. R. Padmanabhan and T. T. Tchen, J. Bact. 100, 398 (1969).

2. J. Tobari and T. T. Tchen, J. Biol. Chem., in press.

3. C. L. Rosano and C. Hurwitz, Biochem. Biophys. Res. Comm., 37, 677 (1969).

4. C. W. Tabor and L. G. Dobbs, J. Biol. Chem., 245, 2086 (1970).

5. I. Pastan and R. Perlman, Science, 169, 339 (1970).

6. U. Bachrach, Ann. Rev. Microbiology, 24, 109 (1970).