SHORT COMMUNICATIONS

Product Induction in the Degradation of Pantothenate in Pseudomonas fluorescens P-2

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INTRODUCTION

Pseudomonads metabolize many organic compounds through inducible pathways. Many of these induction processes require multiplication and protein synthesis de novo, exhibit product induction and are subject to catabolite repression; enzymes are diluted out upon removal of the inducer (Rosenfeld & Feigelson, 1969).

Pseudomonas fluorescens P-2 contains an inducible amidase, pantothenate hydrolase, which hydrolyses pantothenate to pantoate and β-alanine (Nurmikko et al. 1966). The synthesis of this hydrolase is closely linked with cell division, starting at the beginning of the acceleration phase of growth and continuing throughout the active growth phases. Some rapidly metabolized amino acids, such as glutamic acid, glutamine, proline, alanine and β-alanine, as well as several α-keto acids, decrease the formation of the hydrolase when the nitrogen supply is limited (Mäntälä & Nurmikko, 1970).

This communication reports some experiments on the induction of pantothenate hydrolase.

METHODS

[1-14C]-D-Pantothenate (specific activity 4·75 mCi/m mole) was purchased from the New England Nuclear Corporation, Boston, Massachusetts, U.S.A. D-Pantolactone was obtained from K & K Laboratories, Plainview, New York, and D-pantothenate from Fluka AG, Buchs, Switzerland. Aldopantoate and 3,3-dimethylmalate were prepared by a method described by Goodhue & Snell (1966b), except that 18% (w/v) NaOH was used, and aldopantoate was isolated as the calcium salt by stirring with CaCl₂. Nuclear magnetic resonance (n.m.r.) spectroscopy was used to test the purity of the compounds.

Pseudomonas fluorescens P-2 (earlier described as Pseudomonas P-2 by Goodhue & Snell, 1966a) was used as test organism.

Precultivations were carried out in the medium described earlier by Goodhue & Snell (1966a), with glucose or β-alanine as a source of carbon and energy and (NH₄)₂SO₄ as a source of nitrogen. KH₂PO₄ used was 10 mM. The methods of cultivation were the same as described elsewhere (Mäntälä & Nurmikko, 1970).

Pantothenate hydrolase was assayed by the method of Mäntälä & Nurmikko (1970) with some modifications. Samples of 100 μl. were withdrawn at intervals of 2 h. and washed once with 2 ml. of 0·05 M phosphate buffer (pH 7·5). The enzyme was released from the cells by freezing and thawing four times in 100 μl. of 0·05 M phosphate buffer (pH 7·5).
RESULTS

Pantothenate hydrolase was induced in the presence of its substrate, pantothenate, and in the presence of its products, pantoate and aldopantoate (Fig. 1). The next intermediate, 3,3-dimethylmalate, caused neither growth nor induction, and 2-oxoisovalerate and β-alanine, although they were good sources of carbon and energy, had no inductive effect upon the synthesis of the hydrolase.

Several other compounds were tested as inducers of pantothenate hydrolase. Although acetamide, acetate, glucose, glycerol, glycine, oxalacetate and α-oxobutyrate supported growth neither these compounds nor glyoxylate, hydroxypantothenate, panthenol or pantetheine induced pantothenate hydrolase.

3,3-Dimethylmalate only slightly increased the growth of Pseudomonas fluorescens P-2 when precultivated on β-alanine or glucose. Two negative charges in the molecule made the penetration of 3,3-dimethylmalate by diffusion very difficult. However, in cultures precultivated on pantoate or pantothenate short growth periods were observed, suggesting that at least a small amount of this compound entered the bacteria and was further metabolized.

When cultured in a pantothenate medium, the growth phase of Pseudomonas fluorescens P-2 had remarkable effects upon the utilization of substrates. Bacteria taken from the acceleration phase or from the beginning of the exponential phase utilized β-alanine preferentially and prevented the uptake or metabolism of pantoate, aldopantoate and 3,3-dimethylmalate. Bacteria from a later stage of the exponential phase preferred to utilize pantoate and a long lag phase was seen in the β-alanine medium (Mäntisälä & Nurminikko, 1970).
Specific activity reached the maximum level in the pantothenate medium during the late exponential phase, while in the pantoate medium the maximum was reached earlier. The later maximal specific activity in the pantothenate medium can be supposed to be due to the formation of β-alanine, which had a diluting and repressive effect upon the formation of the hydrolase. Furthermore, glucose decreased the formation of pantothenate hydrolase and no increase in specific activity was observed when the concentration of glucose (4 mM) was high compared with that of pantoate (0.5 mM).

**Discussion**

Product induction similar to that observed in the pantothenate system occurs in the mandelate, tryptophan and ρ-hydroxybenzoate pathways of Pseudomonas species (Palleroni & Stanier, 1964; Bird & Cain, 1968; Rosenfeld & Feigelson, 1969) in the histidine system of *Aerobacter aerogenes* (Schlesinger, Scotto & Magasanik, 1965) and Pseudomonas species (Lessie & Neidhardt, 1967) and in the arginine degradation system of *Bacillus licheniformis* (Laishley & Bernlohr, 1968). The significance of this kind of induction is not completely understood, but Palleroni & Stanier (1964) have proposed that an excess of primary substrate over that required for biosynthetic activity can be converted to the inducer, but with a lag such that only a sustained excess of substrate will evoke notable induction. However, the induction of pantothenate hydrolase was observed in the presence of small amounts of inducer. Because β-alanine is the sole source of nitrogen and the inducer of β-alanine-pyruvate transaminase (Goodhue & Snell, 1966a), it is economic for the bacteria that both the inductions of nitrogen metabolism and pantoate degradation can start at the same time and with equal amounts of inducers.

The fact that both panthenol, pantetheine and hydroxypantothenate were wholly ineffective as inducers gives further support to the view that the free pantoate moiety is the real inducer. Aldopantoate, which resembles pantoate, has the ability to induce, too, or is converted to pantoate during cultivation.

Findings in this investigation and those concerning pantoate, aldopantoate and 3,3-dimethylmalate dehydrogenases (unpublished data) suggest co-ordinative control in the pantothenate degradation, where one intermediate, pantoate, induces pantothenate hydrolase, which produces it, as well as the enzymes which decompose it. Furthermore, these inductions parallel each other in various conditions. 2-Oxoisovalerate, which was unable to induce pantothenate hydrolase, although it produced rapid growth, may be a substrate of a semi-sequential step or the next operon.

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**References**


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