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On the Regulation of the Formation of the Pantothenate Transport System during the Growth of *Pseudomonas fluorescens* P-2

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The formation of the pantothenate transport system in *Pseudomonas fluorescens* P-2 was investigated. The transport system was formed inducibly on pantothenate, pantoate, and aldopantoate, but only slightly on 2-oxoisovalerate and not at all on 3,3-dimethylmalate. The results suggest coordinative induction with pantothenate hydrolase.

The pantothenate transport system was also formed on glutamate, glucose, pyruvate, glycerol, and the intermediates of the Krebs cycle. β-Alanine, propionate, pyruvate, and glucose decreased the formation of the pantothenate transport system induced by pantoate, suggesting that two independent transport systems are linked with pantothenate transport.

Glyoxylate, glycolate, and formate, in the presence of a nitrogen source and glycine, activated pantothenate uptake. The possible role of pantothenate uptake in the control of pantothenate degradation was discussed.

Active transport systems for many compounds have been demonstrated in a variety of microorganisms. Attempts have been made to establish the relationship between substrate transport and other metabolic activities. Some of these uptake systems have been found to be inducible [1–5] or repressible,[6–9] the others being constitutive.[10–13]

Some of these transport activities are coordinatively controlled with other enzyme activities in the pathway. Others, for example galactose [14] and arabinose [15] permeases in *Escherichia coli*, tryptophan permease in *Pseudomonas acidovorans*,[16] and malate permease in *Streptococcus faecalis*,[9] appear not to be linked with the next enzymes along the pathways. On the other hand, β-galactoside permease and β-galactosidase in *Escherichia coli*,[17] glutamate transport and glutamate oxidation in *Mycobacteria*,[2] and α-glucoside transport and α-glucosidase in *Saccharomyces cerevisiae* [5] have been found to be coordinatively controlled.

These and other examples of the activity control suggest general patterns of enzyme regulation in the transport processes.

This report concerns the control of the pantothenate uptake system in *Pseudomonas fluorescens* P-2. The transport system was formed inducibly in the presence of pantothenate, pantoate, and aldopantoate, but only slightly in a medium containing 2-oxoisovalerate, and not at all in one containing 3,3-dimethylmalate.

The transport system for pantothenate was also formed, noncoordinatively in the presence of glutamate, glucose, pyruvate, glycerol, and the intermediates of the Krebs cycle.

Glyoxylate, glycolate, and formate, in the presence of a nitrogen source, activated the pantothenate transport system.

EXPERIMENTAL

Materials. The chemicals were those described in the text of the preceding paper.[18] Aldopantoate and 3,3-dimethylmalate were prepared as described earlier.[19,20]

Cultivations. Pseudomonas fluorescens P-2 was used in transport studies. The cultures were aerated as described elsewhere.[21,22] The concentration of each carbon source was 10 mM. The cells (0.17 mg of dry weight) were collected in a refrigerated centrifuge (5000 *g*, 10 min) and washed with cold 0.05 M phosphate buffer (pH 7.5).

Uptake assay. Transport activity was measured as described in the preceding paper.[18]

RESULTS

As can be seen from Fig. 1, formation of the pantothenate uptake system was closely linked with the growth cycle of *Pseudomonas fluorescens* P-2 in the pantothenate, pantoate or aldopantoate media. Formation of the transport system started when growth was at the beginning and in each medium the specific activity reached maximum levels during the exponential phase. However, maximum levels were reached earlier in the pantoate medium than in the pantothenate medium and the maximum was also higher in the pantoate medium. When bacteria were precultured for two generations in the β-alanine medium and then transferred to the media containing different amounts of pantoate (0.5 – 10 mM), a lag phase of 3 – 4 h was observed (Fig. 2). In the presence of 5 and 10 mM pantoate the rise in transport activity was about 10-fold compared with the activities of the lag phase.

On 3,3-dimethylmalate, 2-oxoisovalerate, and β-alanine, which are the intermediates of pantothenate degradation,[19–22] formation of the pantothenate uptake system was almost negligible, although 2-oxoisovalerate and β-alanine caused rapid growth (Fig. 3).

Many other carbon compounds were tested as possible inducers of pantothenate uptake. Fig. 4 shows the results of these experiments. As we can see, the pantothenate uptake system was formed on almost all compounds investigated, although the activity levels were negligible compared with those reached in the pantoate, pantothenate, and aldopantoate media. Furthermore, no sharp rise in specific activity was observed.

When bacteria were cultured on glyoxylate, glycolate, glycine or formate, the specific activities rose strongly (Fig. 5). Especially on glyoxylate the rise in transport activity was very rapid, reaching a maximum in 3 – 4 h, although

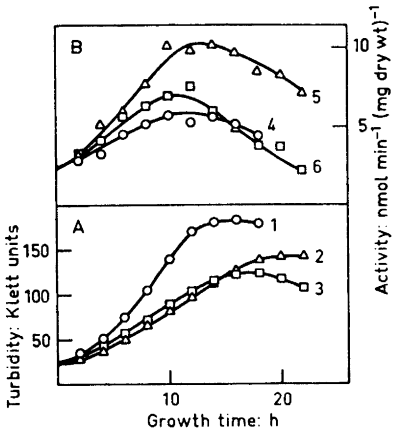

Fig. 1. Effect of pantothenate (10 mM), pantoate (10 mM) and aldopantoate (20 mM) on the formation of the pantothenate transport system in *Pseudomonas fluorescens* P-2. The reaction mixture (0.2 ml of salt solution [21]) contained 0.1 μmol of K-pantothenate, 40 000 cpm of Na-[^{14}C]-pantothenate (specific activity 4.75 mC/mmol), 0.17 mg of dry weight of bacteria and 20 μg chloramphenicol. The reaction was stopped after incubation for 1 min at 30° by adding 1 ml of ice-cold 0.15 M sodium chloride containing 10 mM K-pantothenate. The samples were immediately washed on a Millipore filter (0.30 μm) for 2 min with the same ice-cold salt solution. The filters were counted in a liquid scintillation spectrometer. A, Turbidity of the culture; 1 = pantothenate; 2 = pantoate; 3 = aldopantoate. B, Specific activity of the transport system; 4 = pantothenate; 5 = pantoate; 6 = aldopantoate.

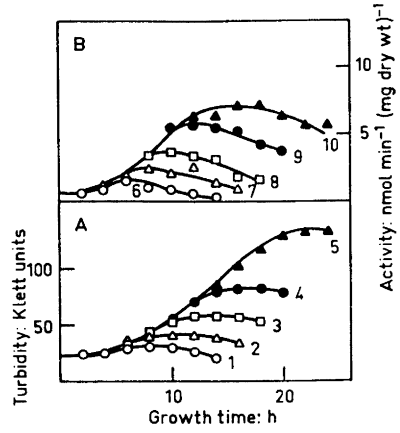

Fig. 2. Effect of different pantoate concentrations on the length of the lag phase in growth and transport activity. The experimental conditions were those described in the text of Fig. 1. A, Turbidity of the culture; 1 = 0.5 mM; 2 = 1 mM; 3 = 2 mM; 4 = 5 mM; 5 = 10 mM pantoate. B, Specific activity of the transport system; 6 = 0.5 mM; 7 = 1 mM; 8 = 2 mM; 9 = 5 mM; 10 = 10 mM pantoate.

growth was very slow. In the glycolate, glycine, and formate media, maximum activity was reached somewhat later, suggesting that they do not produce so much energy for the transport system or that they are converted more slowly to active compounds than glyoxylate. Chloramphenicol had no marked effect on this "induction", suggesting that no new protein synthesis is linked with this process. However, when bacteria were cultured in media devoid of nitrogen, no increase in pantothenate uptake activity was seen in the glyoxylate medium (Fig. 5). Acetate only slightly increased the formation of the pantothenate uptake system.

Because β-alanine, pyruvate, glutamate, and glucose had been shown to repress pantothenate hydrolase,[20,22] they were also tested as possible repressors of pantothenate uptake, using pantoate (Fig. 6) and glyoxylate (Fig. 7)

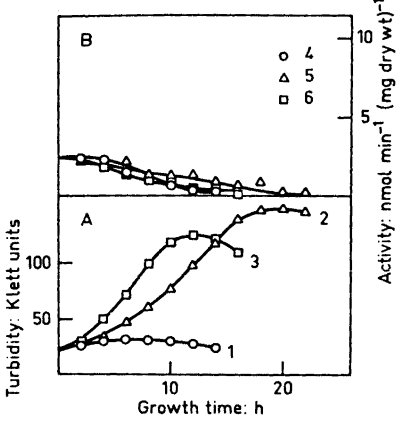

Fig. 3. Formation of the pantothenate transport system in the presence of 3,3-dimethylmalate (20 mM), 2-oxoisovalerate (20 mM), and β-alanine (20 mM). The experimental conditions were those described in the text to Fig. 1. A, Turbidity of the culture; 1 = 3,3-dimethylmalate; 2 = 2-oxoisovalerate; 3 = β-alanine. B, Specific activity of the transport system; 4 = 3,3-dimethylmalate; 2 = 2-oxoisovalerate; 3 = β-alanine.

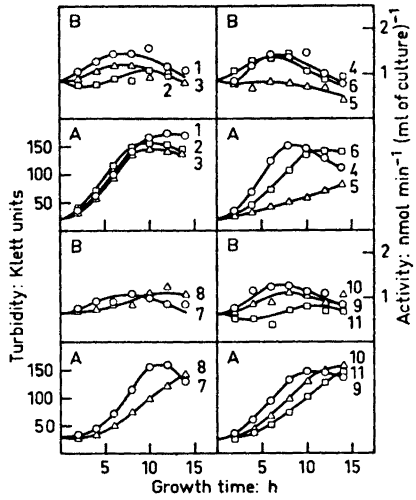

Fig. 4. Formation of the pantothenate transport system on different compounds (20 mM). The experiment was carried out as described in the text to Fig. 1. A, Turbidity of the culture; 1 = citrate; 2 = succinate; 3 = fumarate; 4 = glutamate; 5 = valine; 6 = oxalacetate; 7 = glucose; 8 = glycerol; 9 = pyruvate, 10 = lactate; 11 = propionate. B, Specific activity of the transport system. The symbols are the same as above.

as inducers. When pantoate was the inducer of pantothenate uptake, all the compounds investigated decreased total activity and the effects were most powerful at the beginning of growth. They also decreased pantothenate uptake

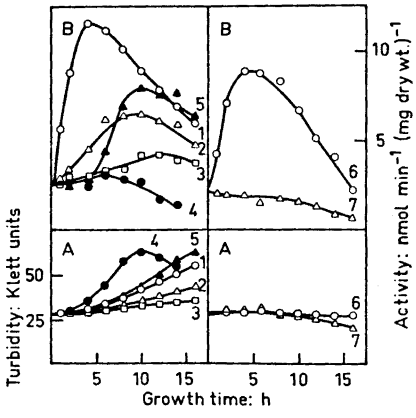

Fig. 5. Formation of the pantothenate transport system on different compounds (20 mM). Experimental details were as described in the lengend to Fig. 1. A, Turbidity of the culture; 1 = glyoxylate; 2 = glycine; 3 = formate; 4 = acetate; 5 = glycolate; 6 = glyoxylate and chloramphenicol (100 μg/ml); 7 = glyoxylate without ammonium sulphate. B. Specific activity of the transport system. The symbols are the same as above.

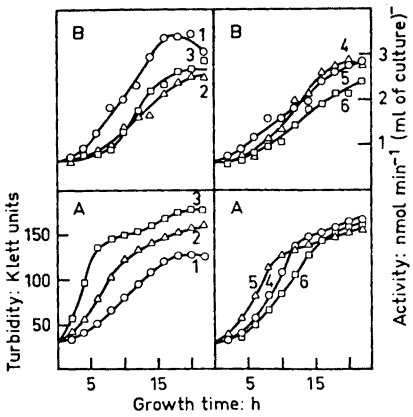

Fig. 6. Effect of different carbon sources (10 mM) on the formation of the pantothenate transport system with pantoate (10 mM) as inducer. The experimental details were those described in the legend to Fig. 1. A, Turbidity of the culture; 1 = pantoate; 2 = pantoate + β-alanine 3 = pantoate + glutamate; 4 = pantoate + glucose; 5 = pantoate + pyruvate; 6 = pantoate + propionate. B, Specific activity of the transport system. The symbols are the same as above.

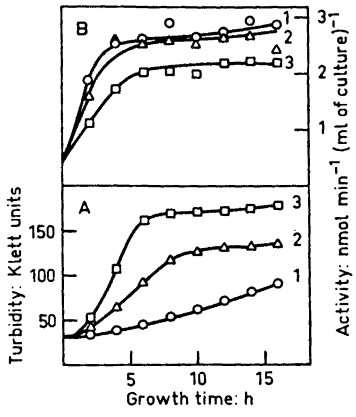

Fig. 7. Effect of β-alanine (10 mM) and glutamate (10 mM) on the formation of the pantothenate transport system with glyoxylate (10 mM) as "inducer". Experimental conditions were as described in the legend to Fig. 1. A, Turbidity of the culture; 1 = glyoxylate; 2 = glyoxylate + β-alanine; 3 = glyoxylate + glutamate, B. Specific activity of the transport system. The symbols are the same as above.

when glyoxylate was used as transport inducer. However, the shape of the specific activity curve remained almost unchanged, suggesting that the decrease in activity was not linked with catabolite repression.

DISCUSSION

These and the other results [20,22] suggest a coordinative control of the pantothenate transport system and pantothenate hydrolase formation in *Pseudomonas fluorescens* P-2. Pantoate and aldopantoate, the first intermediates in the pantothenate degradation pathway, were not only the most powerful inducers of pantothenate hydrolase,[20] but also the most effective inducers of pantothenate uptake.

The physiological role of this kind of product induction is still obscure. However, if we postulate the existence of an operon model where pantoate is a real inducer of pantothenate "permease", pantothenate hydrolase, and pantoate degradation, induction of the enzymes of pantoate and β-alanine metabolism can occur at the same time and at equal concentrations of inducers. This is important, because the amino group of β-alanine, which is the only nitrogen source of pantothenate, is transferred by inducible β-alanine–pyruvate transminase.[23] At low enzyme levels, on the other hand, product induction prevents exhaustion of the substrate.

The effects of 3,3-dimethylmalate, 2-oxoisovalerate, and β-alanine also reflect the coordinative control of the transport of pantothenate and pantothenate hydrolase. However, if the operon model is correct, 3,3-dimethylmalate is hardly likely to have a transport system of its own, and as earlier mentioned [20] two negative charges in the molecule prevent its easy penetration into the cells.

Rosenfeld and Feigelson [16] have found a product induction of tryptophan permease and tryptophan oxygenase in *Pseudomonas acidovorans*. However, these inductions had not been coordinated with each other.

Because of the inducibility of several transport systems we must postulate the existence of a basal permease level in the cells or else that there is another transport mechanism which operates at the same time or accelerates the accumulation of substrates. Several reports suggest that in many catabolic systems, when the inducer is present at high concentrations, some of it enters the cell by diffusion. Precultivations for several generations on β-alanine, propionate, or glucose, decreased both pantothenate hydrolase and pantothenate transport activities almost to zero level. The long lag phase when the cells were transferred to the pantothenate medium can be explained by slow diffusion of pantothenate and low basal levels of pantothenate hydrolase, which prevent formation of the inducer, pantoate.

Because of coordinative control of pantothenate uptake and pantothenate hydrolase, the specific activities were achieved at the same time (Fig. 1).[20] As with many other catabolic enzyme systems,[24–27] neither enzyme reached its maximum activity until the end of the exponential phase. Because pantothenate hydrolase and pantothenate permease are needed throughout all growth phases and because the inducers of both enzyme systems are formed as long as pantothenate hydrolase can act, both enzymes are synthetized in parallel with cell multiplication.

When bacteria were cultivated in the presence of pantoate and β-alanine, glucose, pyruvate, glutamate, or propionate, the specific activity maximum of the permease system was shifted towards the stationary phase and a decrease in total activity was observed. This is in good agreement with the earlier results [22] and provides further evidence for the coordination hypothesis. Earlier reports likewise suggest that the transport systems are sensitive to catabolite repression. For example, malate permease in *Streptococcus faecalis*,[9] maltose uptake in *Saccharomyces cerevisiae*,[28] and tryptophan [29] and fucose [30] uptakes in *Escherichia coli* have been found to decrease in the presence of glucose.

The ability of several metabolites to "induce" pantothenate uptake, whereas the others were fully inactive, is not easy to interpret. However, the increase in transport activity caused by glyoxylate, glycolate, formate, or glycine is not linked with protein synthesis, because this rise in activity could not be prevented by chloramphenicol. On the other hand, absence of a nitrogen source diminished transport activity to the basal level. Glyoxylate, glycolate, formate, and glycine may directly affect the activity of the transport system, although this activation is a slow process and demands a nitrogen source. These results suggest conversion of the metabolites to active forms or liberation of energy for transport. Kaback and Milner [31] and Barnes and Kaback [32]

reported that the conversion of D-lactate to pyruvate markedly stimulated the transport of several amino acids and β-galactosides. According to Britten and McClure,[33] transport systems of all amino acids in *Escherichia coli* and, according to Kay and Gronlund,[34] transport systems of all aliphatic amino acids in *Pseudomonas aeruginosa* decrease as a result of nitrogen starvation. However, this is not the real cause in the case of pantothenate transport, because activity remained at a constant level from the very beginning of nitrogen starvation.

The formation of a pantothenate uptake system in the presence of many other compounds was prevented by chloramphenicol. Furthermore, no pantothenate hydrolase formation was found in the presence of these intermediates (results not given). These and other results described in the preceding paper [18] suggest the existence of another, unspecific, transport system, which is not formed coordinatively with pantothenate hydrolase or else that pantothenate most easily diffuses into the cells from the exponential phase.

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