SHORT COMMUNICATIONS

The Relationship between Methionine Uptake and Demethiolation in a Methionine-utilizing Mutant of *Pseudomonas fluorescens* UK1

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INTRODUCTION

Attempts to detect demethiolating activity in cell-free extracts have generally been unsuccessful (e.g. Ruiz-Herrera & Starkey, 1970) so that the physiological role and the mode of action of the enzyme is poorly understood. This enzyme has been reported to have a catabolic function in methionine degradation (Segal & Starkey, 1969). However, some organisms can demethiolate methionine without being able to use the amino acid as a carbon source (Ruiz-Herrera & Starkey, 1969) suggesting that the enzyme may play a role in regulating the intracellular concentration of methionine. Therefore, it would be of value to know whether demethiolation is a cytoplasmic reaction or a process by which the cell envelope eliminates excess methionine from the environment.

This paper, based on experiments with intact organisms, demonstrates the dependence of demethiolation on methionine transport.

METHODS

Bacteria. A mutant strain of *Pseudomonas fluorescens* UK1 able to use methionine as the only source of carbon and nitrogen (Mäntsälä, Laakso & Nurmikko, 1974) was used.

Culture media. The carbon and nitrogen sources (10 mM) were dissolved in a basal mineral medium containing (g l⁻¹): KH₂PO₄, 1.35; MgSO₄·7H₂O, 0.26; FeSO₄·7H₂O, 0.003; adjusted to pH 7.2 with KOH.

Growth conditions. The cultures were grown in 100 to 300 ml medium at 30 °C and aerated by bubbling with moist air at a rate of 1.0 l min⁻¹. Exponential growth was reached and maintained by allowing the organism to grow for several hours keeping the turbidity below 50 Klett-readings (filter 62), if necessary by repeated dilutions.

Preparation of washed cell suspensions. Samples (about 2 mg dry wt organisms) were centrifuged at 5000g for 15 min, washed twice with the basal mineral medium, and resuspended in 1.0 ml of the same medium.

Measurement of methionine transport. The reaction mixture contained, in 2.0 ml basal mineral medium, 20 nmol L-[1-¹⁴C]methionine (15.6 mCi mmol⁻¹, New England Nuclear, Boston, Massachusetts, U.S.A.), and 0.4 mg dry wt organisms (Mäntsälä *et al.*, 1974).

Measurement of demethiolation. A spectrophotometric procedure was used. The standard assay mixture contained, in 1.0 ml basal mineral medium, 3 µmol L-methionine, 0.2 µmol 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and 0.25 mg dry wt washed organisms. The increase in extinction at 412 nm was followed using a Pye Unicam SP 800 spectrophotometer. The molar extinction coefficient of the coloured aryl mercaptan was 13 200 l mol⁻¹ cm⁻¹, based on the reaction of DTNB with a standardized solution of L-cysteine at pH 7.2.

Table 1. *Effect of carbon source on methionine transport and demethiolation*

Methionine transport rates and demethiolase activities were measured as described in Methods.

Carbon source (10 mM)	Initial velocity [nmol min ⁻¹ (mg dry wt organisms) ⁻¹]	
	Transport	Demethiolation
DL-Methionine	2.75	2.09
2-Oxobutyrate	5.23	0.22
Propionate	8.66	0.51
Succinate	9.90	0.31
Glucose	13.06	0.11
Glutamate	9.35	0.22
DL-Leucine	5.36	0.44
DL-Alanine	10.86	0.09
DL-Threonine	7.29	0.34
Casamino acids	8.53	0.60

RESULTS

Neither methionine-transporting membrane vesicles, prepared by the method of Stinnett, Guymon & Eagon (1973), nor the corresponding cytoplasmic fraction were able to demethiolate methionine. The relationship of methionine transport to its demethiolation was therefore studied using intact organisms.

To determine whether the organism was able to demethiolate methionine without transporting it, azide (10 mM) and malonate (20 mM), known to inhibit methionine transport (Mäntsälä *et al.*, 1974), were added to washed suspensions of the mutant *P. fluorescens* UK1. However, both processes were inhibited. Attempts to obtain mutants able to demethiolate methionine, but not to transport it, were also unsuccessful. In contrast, addition of chloramphenicol or rifampicin completely inhibited demethiolating activity although the organism was still able to transport methionine. These preliminary results suggested that whenever transport was inhibited so was demethiolation, but inhibition of demethiolation did not necessarily lead to inhibition of transport.

When the organism was grown on different carbon sources, and transport and demethiolating activities were determined in washed suspensions, initial velocities of transport were usually 10 to 100 times higher than the corresponding rates of demethiolation (Table 1). Only in organisms grown on methionine as carbon source did both reactions proceed at similar rates, when, assuming demethiolation is an intracellular process, nearly all the methionine taken up would be demethiolated. A reduction in the transport rate in methionine-grown organisms should cause a parallel inhibition of demethiolation if the enzyme activity is intracytoplasmic. Different rates of methionine transport were achieved by supplementing the standard assay mixtures with non-sulphur amino acids competing with methionine for the same permease. The K_m for methionine transport was 25 μ M and the following K_i values (μ M) for the competing amino acids were obtained: leucine, 30; tyrosine, 51; phenylalanine, 70; isoleucine, 81; threonine, 90; tryptophan, 104; alanine, 111; valine, 129; histidine, 380. In the presence of these amino acids the rates of demethiolation were indeed reduced in parallel with transport rates (Fig. 1). In contrast, glutamine, lysine and aspartate, whilst stimulating methionine uptake, did not enhance the rate of demethiolation over the control level.

Fig. 1. Dependence of demethiolation on methionine transport. Washed suspensions of *P. fluorescens* UK1 mutant grown on methionine as carbon source were used to determine the rates of transport and demethiolation of methionine in the presence of different amino acids. The standard assay mixtures (see Methods) were supplemented with 3 μ mol (demethiolation) or 0.02 μ mol (transport) of the amino acid under investigation. The activities without amino acid supplementation were taken as 100 %.

DISCUSSION

The liberation of enzyme activity from intact protoplasts is generally considered sufficient proof for the existence of an enzyme outside the cell membrane (Dunlop & Roon, 1975). However, the disappearance of demethiolating activity during protoplast formation made such observations impossible and so we used the broad specificity of the permease found previously in mammalian cells, yeasts and moulds (Oxender & Christensen, 1963; Pall, 1969; Benko, Wood & Segel, 1967) and in the *P. fluorescens* UK1 mutant by us. The sensitivity of demethiolation to reductions in the methionine transport rate suggests that the former is an intracellular reaction preceded by the entry of methionine via the permease. One might expect that if demethiolation were a function of the permease itself, the amino acids used to inhibit transport would also be competitive inhibitors of demethiolation. However, the facts that the ratio between the two activities is not constant during growth on different carbon sources and that the activities show different sensitivities to inhibitors of protein synthesis suggest that this is not the case. The fact that demethiolating activity was not increased over the control level with any of the supplemented amino acids (Fig. 1) indicates that even in organisms grown on methionine the rate of demethiolation was not regulated by the activity of the permease.

We are now attempting to characterize factors regulating demethiolating activity in order to define its physiological role.

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