S-ADENOSYLMETHIONINE BIOSYNTHESIS IN *MYXOCOCCUS XANTHUS*

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1. Introduction

Myxococcus xanthus is a Gram-negative bacterium with an interesting developmental cycle. Under nutritionally limiting conditions multicellular fruiting bodies are formed which contain refractile myxospores. Amino acids of the aspartate group affect both colonial and cellular morphogenesis [1,2] and methionine will inhibit fruiting even when all other amino acids are absent [1,2]. Myxospore formation can also be induced outside of the fruiting body by methionine starvation [3] or by a range of hydroxylated compounds including glycerol and phenethyl alcohol [4]. Glycerol does not inhibit methionine uptake nor its incorporation into protein [5] although phenethyl alcohol does inhibit amino acid transport [6].

An explanation of the effects of methionine on development was attempted [2,3,7] by proposing that methionine depletion results in a reduced level of spermidine in the cell. This, in turn, depresses aspartokinase and results in blockage of cell-wall growth through limitation of *meso*-diaminopimelic acid for peptidoglycan synthesis [2]. More recent data indicate that incorporation of *meso*-diaminopimelic acid is stimulated during myxospore formation [8] and therefore this theory is no longer tenable.

Further work is required to establish whether there is a specific role for methionine in the control of cell or colonial differentiation. The experiments described here concern the biosynthesis and cellular levels of *S*-adenosylmethionine in *M. xanthus*.

2. Materials and methods

Myxococcus xanthus (NCIB 9412) was grown in 1% Casitone medium [8]. Log phase cells were disrupted by sonication in Tris–HCl buffer (0.05 M, pH 8) containing 2-mercaptoethanol (2 mM) and $MgCl_2$ (5 mM), at 0–5°C until ~95% cell breakage (determined microscopically). Unbroken cells were removed by centrifugation (5000 × *g*, 5 min) and the supernatant used directly (whole sonicate).

S-Adenosylmethionine synthetase was assayed by a modification of the method in [9]. Whole sonicate (0.5–2 mg protein) was incubated at 30°C in 250 μl total vol. containing 12.5 μmol Tris–HCl (pH 8), 12.5 μmol KCl, 1.25 μmol ATP, and varying L-[*methyl*-^{14}C]methionine. Samples (50 μl) were removed at intervals and mixed with 50 μl acetic acid (1 M) in a plastic centrifuge tube. After standing in ice for 20 min, the mixture was centrifuged (30 s, Micropan 320 Microfuge) and 20–50 μl of the supernatant applied to Whatman no. 1 paper. The chromatogram was developed in *n*-butanol:acetic acid:water (2:1:1, by vol.) and the area corresponding to *S*-adenosylmethionine cut out and counted.

Total cellular pool of *S*-adenosylmethionine was measured in washed exponential phase or glycerol-induced [4] cells. Pelleted cells (4 g dry wt) were extracted with 10 ml perchloric acid (1.5 M) and the *S*-adenosyl-methionine recovered from the neutralised extract by chromatography on Dowex 50-Na^+ [10]. The pool was also examined semi-quantitatively by labelling log phase cells (~2×10^8 cell ml^{-1}) with L-[*methyl*-^{14}C]methionine (1 μCi/ml) in growth medium for 1 h, and extracting the filtered cells with 70% ethanol [11]. Labelled pool material was separated by paper chromatography using *n*-butanol:acetic acid:water (2:1:1, by vol.) solvent system. L-[*methyl*-^{14}C]methionine (60 μCi/μmol) and *S*-adenosyl-L-[*methyl*-^{14}C]methionine (57.2 μCi/μmol) were used as standards (Radiochemical Centre, Amersham).

3. Results and discussion

S-Adenosylmethionine synthetase (ATP-L-methionine *S*-adenosyl-transferase EC 2.5.1.6) was readily detectable in whole sonicates of *M. xanthus*. The enzyme was, however, relatively unstable on storage at 4°C or −20°C. Although some protection of enzyme activity was achieved by inclusion of Mg^{2+} (as 5 mM $MgCl_2$ or $MgSO_4$) in the sonication buffer, all assays were performed in <3 h of cell disruption.

In the absence of inhibitors the enzyme was observed to have a K_m 2.5×10^{-4} M for methionine. At low (0.05 mM) methionine concentrations the enzyme was susceptible to inhibition by cycloleucine (fig.1), and the degree of inhibition was similar to that reported in *Escherichia coli* [9]. Between 0.1–10 mM cycloleucine the inhibition was proportional to the log cycloleucine concentration (I_{50} 1 mM). At higher (1 mM) levels of methionine the enzyme was not significantly inhibited (fig.2) and at 5 mM methionine some stimulation of activity by cycloleucine has been observed. This lack of inhibition by cycloleucine at high substrate concentrations is a feature shared with the *S*-adenosylmethionine synthetase from rat liver

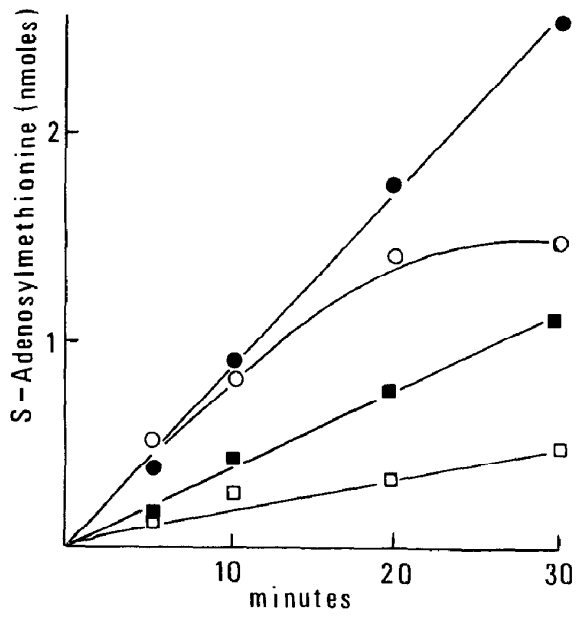

Fig.1. Effect of inhibitors on *S*-adenosylmethionine synthetase. Whole sonicate (1 mg protein) was incubated with 0.05 mM methionine as in section 2: (•) Control; (○) + 0.5 M glycerol; (■) + 1 mM cycloleucine; (□) + 0.5 mM *S*-adenosylmethionine.

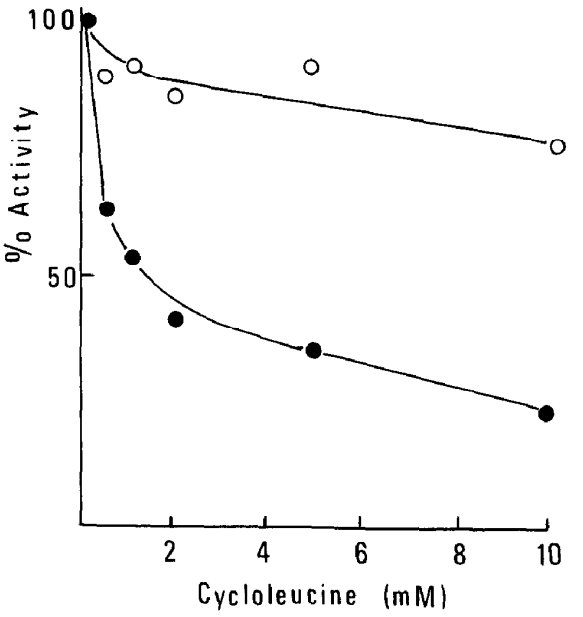

Fig.2. Cycloleucine inhibition of *S*-adenosylmethionine synthetase at different methionine concentrations: (○) 1 mM methionine; (•) 0.05 mM methionine.

but not seen in *E. coli* [9]. The functional significance of this property is not known [9].

Low levels of the product (*S*-adenosylmethionine) have been reported to activate the synthetase [12,13] but when this was examined in preparations from *M. xanthus* only inhibition of activity was observed (fig.1). Between 0.05–1.0 mM methionine the inhibition was uncompetitive (fig.3) (K_i 10^{-4} M *S*-adenosylmethionine). To examine whether this finding was of significance in respect to the control of methionine metabolism, the pool of *S*-adenosylmethionine in the cell was measured.

Total amounts of *S*-adenosylmethionine were determined in vegetative cells to be 0.47 (±0.03) μmol/g dry wt (av. 3 separate cultures). On incubation with the myxospore inducer, glycerol, for 1 h prior to extraction, the pool was reduced to 0.28 (±0.04) μmol/g dry wt. Glycerol induction thus reduced the pool by 41%. The effect of glycerol on *S*-adenosylmethionine synthetase was investigated to determine whether the reduction in the pool could be the result of lower enzyme activity. The total activity was similar in sonicates prepared from vegetative or glycerol treated cells. Glycerol was found to have no effect on the initial rate of *S*-adenosylmethionine synthesis (fig.1) but gradually inhibited *S*-adenosyl-

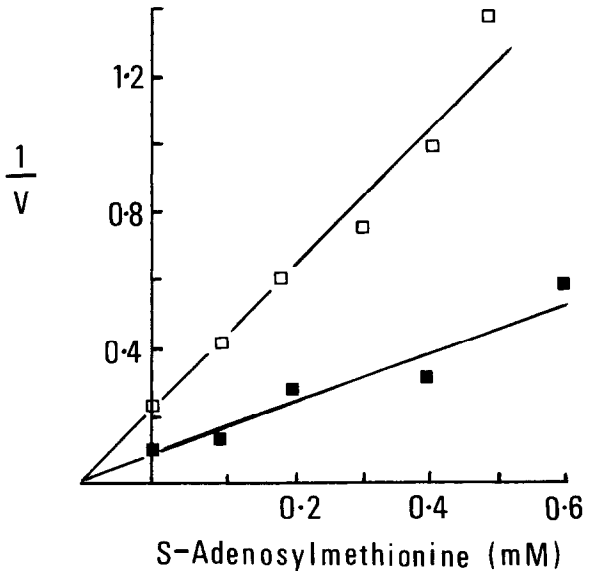

Fig.3. Product inhibiton of *S*-adenosylmethionine synthetase. Dixon plot ($1/v$ versus [*S*-adenosylmethionine]) at different substrate concentrations: (□) 0.05 mM methionine; (■) 0.2 mM methionine. Velocity = nmol *S*-adenosylmethionine. h^{-1} . mg whole sonicate protein^{-1}.

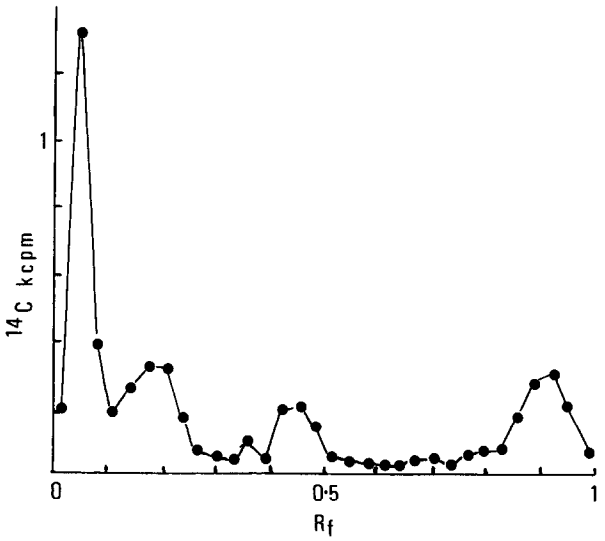

Fig.4. Chromatographic profile of L-[*methyl*-^{14}C]methionine-labelled pool material. Vegetative cells were incubated in growth medium with 1 μCi/ml methionine for 1 h prior to filtration and extracted with cold 70% (v/v) ethanol.

methionine formation and in some instances resulted in a decrease in the amount of product already formed. The effects were variable and at present are best explained by glycerol stimulating the breakdown of *S*-adenosylmethionine (unpublished).

The size of the *S*-adenosylmethionine pool relative to that of methionine was also examined. Vegetative cells were incubated in growth medium with L-[*methyl*-^{14}C]methionine and the ethanol-extracted pool components separated by paper chromatography. Four major peaks at R_F 0.05, 0.2, 0.46, and 0.92 were found to be labelled with ^{14}C (fig.4). The peaks at R_F 0.05 and 0.46 co-chromatographed with *S*-adenosylmethionine and methionine standards, respectively. As in some other Gram-negative bacteria [11] the pool of *S*-adenosylmethionine was several times greater than that of methionine itself (fig.4). This is of considerable importance in view of our finding that *S*-adenosylmethionine is a strong inhibitor of its own synthesis (fig.3). The relatively low affinity of the synthetase for the substrate, methionine, together with the small pool of methionine in the cell would mean that the synthesis of *S*-adenosylmethionine should respond rapidly to methionine depletion. Methionine starvation, but not glycerol addition, resulted in a lower cellular level of spermidine [3]. Since myxospore formation can be induced by glycerol [4] or by methionine starvation [3], this could be interpreted as showing that the fall in the *S*-adenosylmethionine pool following glycerol induction may be of greater significance to the control of cellular differentiation than the polyamine level. However, more needs to be learnt, particularly concerning the breakdown of *S*-adenosylmethionine in *M. xanthus*, before any firm conclusions can be reached about the role of methionine in myxospore formation.

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