THE UPTAKE OF *S*-ADENOSYL-L-METHIONINE IN THE AQUATIC FUNGUS *ACHLYA AMBISEXUALIS*

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Received 11 November 1981

1. Introduction

The thionium compound SAM is synthesized by the transfer of an adenosyl group from adenosine triphosphate to the sulfur atom of methionine; reaction is catalysed by methionine adenosine transferase (EC 2.5.1.6). The activated methyl group of its methionine moiety makes SAM a major methyl group donor for methylation reactions involving nucleic acids, proteins, phospholipids and carbohydrates. Although the metabolic function of endogenous SAM is understood to some extent in several systems [1–4], many cells appear to be incapable of transporting exogenous SAM [4–6]. The existence of a specific SAM uptake system has to our knowledge been reported only for the yeast *Saccharomyces cerevisiae*, where the compound is rapidly accumulated intracellularly at the expense of metabolic energy [7]. Since the water mold *Achlya ambisexualis* is positively chemotropic to SAM, and methyl groups from exogenously supplied SAM are incorporated into proteins and phospholipids of this organism (D. T., E. M., in preparation), we have investigated the nature of SAM permeation in this aquatic fungus.

2. Materials and methods

2.1. *Organism*

Achlya ambisexualis J. R. Raper (male strain E 87) was used. Cultures were grown in PYG medium (peptone, 1 g; yeast extract, 1 g; glucose, 3 g/l distilled water; 100 ml/250 ml Erlenmeyer flask) from encysted spores (fresh or stored at 4°C; ~1×10^4/100 ml medium) for 24 h at room temperature on a reciprocating shaker (Eberbach, 90 cycles/min). Cultures reached the mid-logarithmic phase of growth within 24 h; mycelia from the logarithmic phase were used to prepare spores.

Abbreviations: SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; PYG, peptone, yeast extract, glucose; 2,4-DNP, 2,4-dinitrophenol; CCCP, carbonylcyanide–*p*-chlorophenylhydrazone; SHAM, salicylhydroxamic acid

2.2. *Preparation of spores and germlings*

The method used was modified from that in [8]; mycelia were harvested by filtration, washed 3 times with 250 ml each of 5×10^{-4} M $CaCl_2$, transferred to a 2800 ml Fernbach flask containing 1 liter of 5×10^{-4} M $CaCl_2$ and incubated at room temperature with gentle agitation (Eberbach reciprocating shaker) at 60 cycles/min to facilitate sporulation, which took 6–8 h; spores were separated from mycelia by aseptic filtration through a nylon mesh (25 μm mesh size) and concentrated by millipore filtration (Nalgene; 0.45 μm pore size). Germlings (germinated spores with short germ tubes) were obtained by incubating spores in PYG medium for 6 h with gentle agitation at room temperature. The germlings were washed with and resuspended in Tris–glucose buffer (0.01 M Tris; 7.5 mM glucose, pH 6.9) at 1×10^6 germlings/ml (~65 μg protein/ml) for transport studies. The germling density was determined by hemocytometer count; protein determination was as in [9].

2.3. *Transport assay*

SAM uptake by germlings was measured using a procedure in [10] for amino acid-transport studies. Germlings (6 h old) were suspended in a medium composed of 0.01 M Tris and 7.5 mM glucose (pH 6.9) (except in pH studies). The suspension (1 ml, ~65 μg protein/ml) was incubated with various concentrations of SAM containing either *S*-adenosyl-L-[*methyl*-^{14}C]methionine or *S*-adenosyl-L-[*methyl*-

Published by Elsevier/North-Holland Biomedical Press
00145793/82/0000–0000/$02.75

[^{3}H]methionine (spec. act. 1.25–5.9 X 10^6 cpm/μmol) for different time intervals at room temperature, ~23°C (except in temperature studies). Uptake was terminated by rapid filtration using Millipore filters (HAWP, 0.45 μm pore size) and washing 3 times with 10 ml aliquots of Tris–glucose buffer. After drying under a heat lamp for 1 h, the filters were transferred to scintillation vials and the radioactivity was determined by liquid scintillation counting. Where applicable, potential inhibitors (amino acids, adenine, adenosine, *S*-adenosyl homocysteine and metabolic poisons) were added to the reaction mixture before the addition of germlings. All experiments have been repeated one or more times in duplicates. Where applicable, the curves were fitted using linear regression analysis.

2.4. *O_2 consumption*

The procedure used was as in [11].

2.5. *Chemicals*

S-Adenosyl-L-[*methyl*-^{14}C]methionine (spec. act. 60 mCi/mmol) and *S*-adenosyl-L-[*methyl*-^{3}H]methionine (spec. act. 72 Ci/mmol) were obtained from Amersham (Oakville ON). All other chemicals were purchased from Sigma Chemicals (St Louis, MO) and were of reagent grade.

3. Results

3.1. *Effects of temperature and pH*

The transport of SAM in *Achlya ambisexualis* is dependent on temperature (fig.1A) and pH (fig.1B). The optimum temperature for transport is between 30–40°C; the Q_{10} for 25–35°C is ~1.8; even at 0°C and 50°C limited uptake takes place. The maximum rate of accumulation was at pH 5, with only moderate reduction of uptake as pH was increased to 7.

3.2. *Kinetics of uptake*

As shown in fig.2 *Achlya* germlings rapidly accumulate SAM. Uptake was linear with time for the initial 5 min, indicating that efflux was negligible during this period; a steady state level was attained within 30 min. The initial linearity of uptake suggests that a

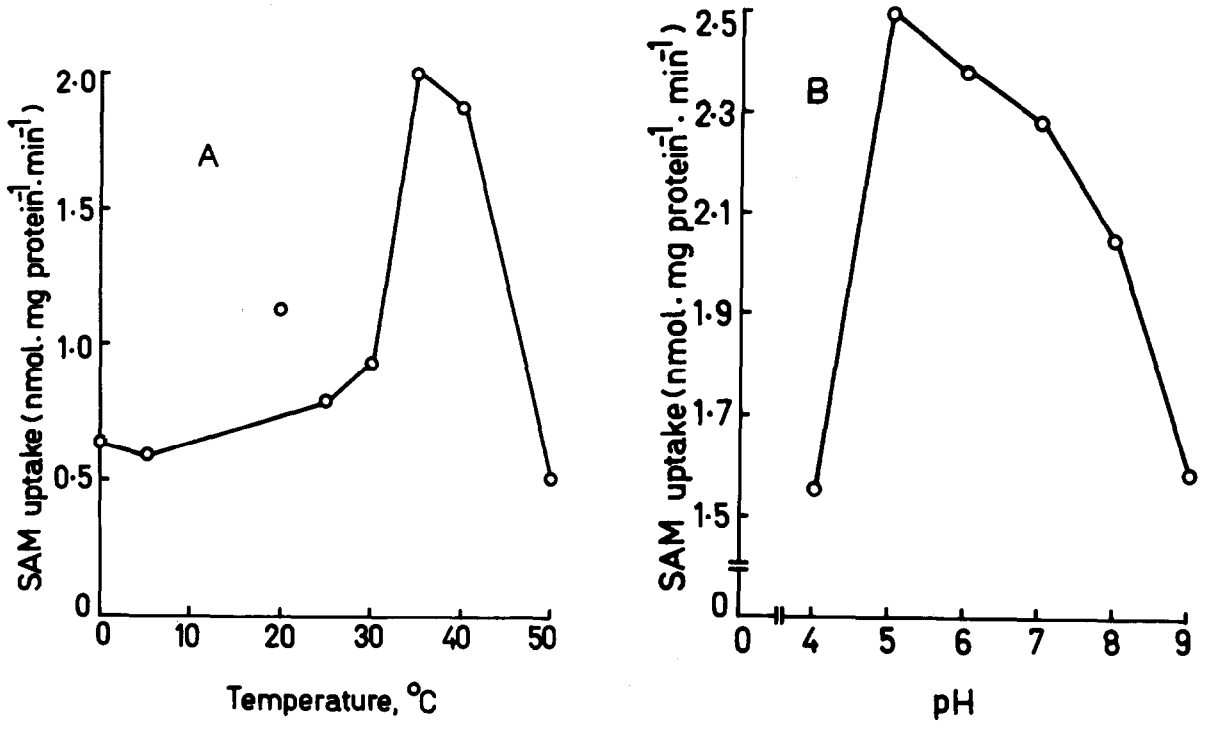


Fig.1. Effect of temperature (A) and pH (B) on SAM uptake in *Achlya ambisexualis* germlings. The initial velocities of uptake at different temperatures and pH values were measured as in section 2. SAM was used at 100 μM.

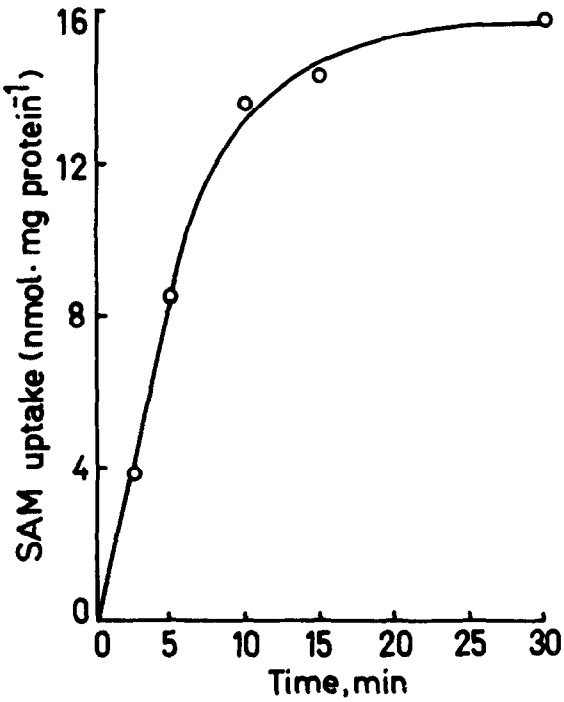


Fig.2. Time course of SAM uptake. Accumulation of SAM for various time intervals was measured as in section 2. SAM was used at 100 μM.

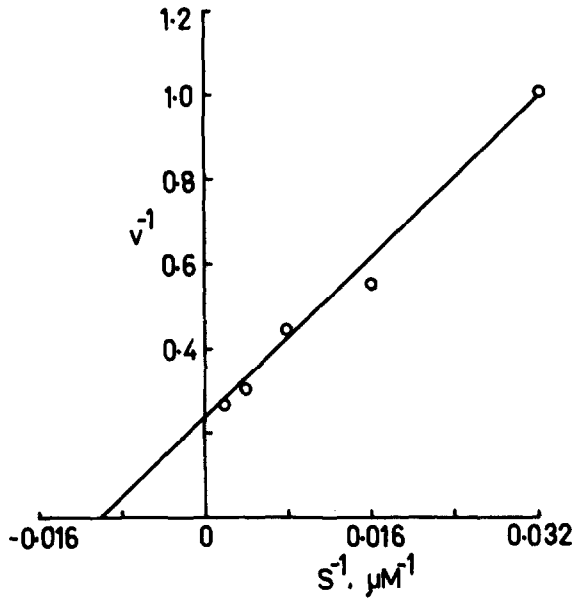
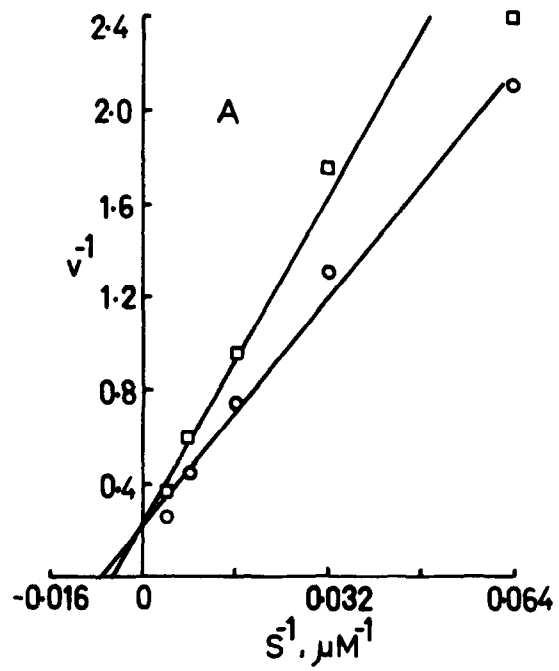


Fig.3. Rate–concentration relationship of SAM uptake: Lineweaver–Burk plot; *s*, SAM conc. (μM); *v*, initial uptake rate (nmol . mg protein^{-1} . min^{-1}).



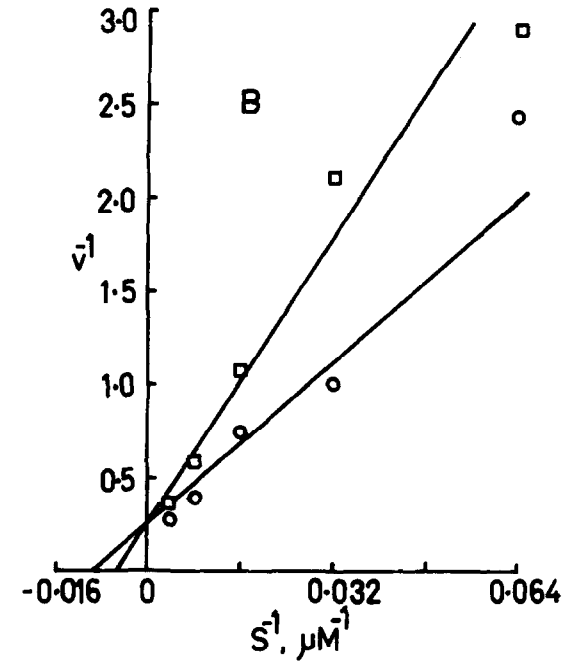


Fig.4. Lineweaver–Burk plot of the inhibition of SAM uptake by cysteine (A) and methionine (B); *s*, SAM conc. (μM); *v*, initial uptake rate (nmol . mg protein^{-1} . min^{-1}); (○–○) control; (□–□) 1 mM cysteine or methionine.

valid measurement of transport velocity is possible within the first 5 min; in subsequent kinetic studies transport was measured for 60 s. Analysis of the rate–concentration relationship indicates that the SAM transport system is substrate–saturable with an app. K_m of 1×10^{-4} M and a V_{max} of 4 nmol SAM . mg protein^{-1} . min^{-1} (fig.3).

3.3. *Specificity of uptake*

The specificity of the SAM-transport system was analysed using potential inhibitors; among amino acids used, leucine and alanine were ineffective (not shown) while cysteine and methionine at 1 mM decreased SAM uptake by 20–30%. Kinetic analysis of the transport data for SAM in the presence of cysteine or methionine (fig.4) showing alteration of the K_m value in each case, indicates competitive inhibition. Inhibition of SAM uptake by adenine and adenosine was ~56% and ~40% percent, respectively. Only the K_m is affected, (fig.5) suggesting competitive inhibition. *S*-Adenosyl homocysteine, a competitive inhibitor of methionine adenosyl transferase not only failed to inhibit SAM transport (fig.6), but actually stimulated uptake.

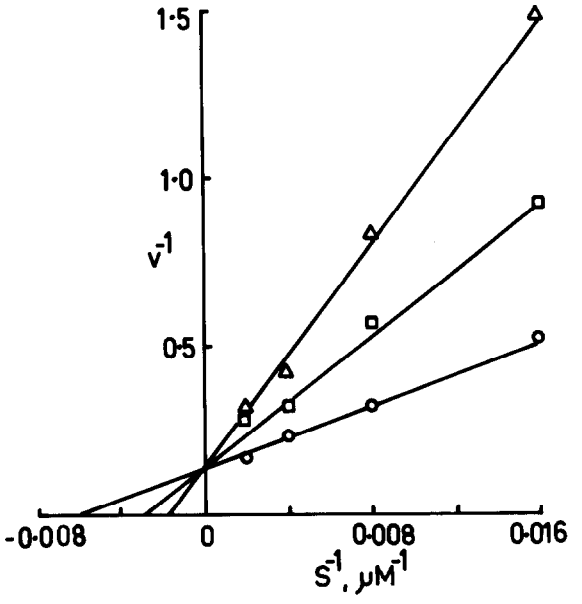


Fig.5. Lineweaver–Burk plot of the inhibition of SAM uptake by adenine and adenosine: *s*, SAM conc. (μM); *v*, initial uptake rate (nmol . mg protein^{-1} . min^{-1}); (○–○) control; (△–△) 1 mM adenine; (□–□) 1 mM adenosine.

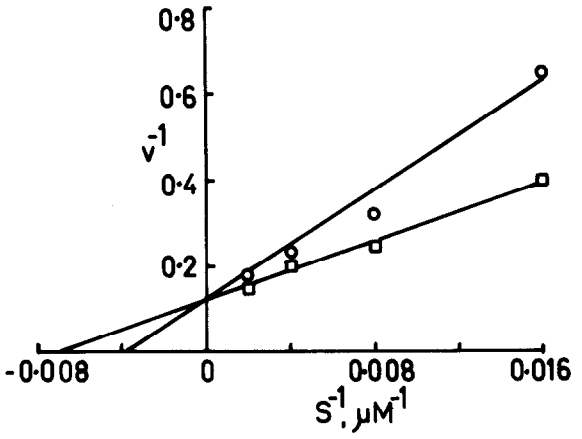


Fig.6. Stimulation of SAM uptake by SAH: Lineweaver–Burk plot; *s*, SAM conc. (μM): *v*, initial uptake rate (nmol . mg protein^{-1} . min^{-1}); (○–○) control; (□–□) 1 mM SAH.

3.4. *Effect of metabolic inhibitors*

The dependence of SAM-transport on metabolic energy was studied using metabolic poisons such as respiratory inhibitors, and uncouplers of oxidative phosphorylation. A summary of the results, together with values for O_2 uptake inhibition, where appropriate, appears in table 1. Sodium cyanide, the most potent respiratory inhibitor used, was the strongest inhibitor of SAM uptake. DNP and CCCP, known to uncouple oxidative phosphorylation and to act as proton conductors across membranes [12], decreased SAM uptake, while SHAM, an inhibitor of a cyanide-resistant 'alternate' respiratory pathway [13], was without effect. Most respiratory inhibitors tested reduced SAM uptake, indicating that the process is energy dependent.

Table 1
Effect of metabolic inhibitors on SAM uptake in *Achlya ambisexualis*

Compound	Conc. (M)	Percent inhibition of:	
		SAM uptake	O_2 utilization
Sodium cyanide	1×10^{-3}	53	77
2,4-DNP	1×10^{-3}	50	n.a.
CCCP	1×10^{-3}	46	n.a.
Iodoacetate	1×10^{-3}	44	40
Sodium azide	1×10^{-3}	27	56
SHAM	1×10^{-3}	0	27

n.a., not applicable

4. Discussion

Dependence of SAM transport on pH and temperature, with Q_{10} for 25–35°C approaching 2, indicates a mediated process rather than simple diffusion. A steady state intracellular concentration of SAM is attained rapidly, within 30 min. In contrast to mediated transport, simple diffusion of this hydrophilic molecule would likely be slow. The transport system is saturable and obeys Michaelis–Menten kinetics. Uptake rate plotted *vs* increasing substrate concentration yielded a rectangular hyperbola rather than a linear response, indicating the presence of a mechanism for mediated uptake, as did competitive inhibition of SAM permeation by cysteine, methionine, adenine and adenosine. Permeation of SAM is dependent on metabolic energy, with metabolic poisons in general exerting strong uptake inhibition.

The temperature optimum for SAM uptake, 35°C, was somewhat higher than that for growth, which in *Achlya* germlings seems to be ~30°C (not shown). Uptake of other substrates, amino acids and glucose, by *Achlya* is optimal at 30°C [10,14]. A temperature of 35°C after growth at 30°C, has been applied to *Achlya ambisexualis* as a heat-shock treatment [15].

Noteworthy was the effect of SAH on SAM uptake, which was stimulatory in *Achlya* in contrast to its inhibitory effect in the yeast system [7].

This report of a specific transport system for SAM is to our knowledge unique for *Achlya* and for an Oomycetous organism. The existence of this mechanism appears puzzling, since under natural growth conditions for a water mold exogenous SAM would not be expected to be available in significant quantities.

Presence of a permeating mechanism for SAM is useful for in vivo studies of the roles of substrate methylation in development. The advantage of exogenous SAM, compared with its precursor, methionine, as a methyl donor, is particularly evident for protein methylation studies, since SAM can be employed without resorting to expedients such as the use of protein synthesis inhibitors.

Acknowledgements

This work was supported by the National Science and Engineering Research Council of Canada. The authors thank Dr Norman F. Taylor for his advice and constructive comments.

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