S-Adenosylmethionine and *Pneumocystis carinii*

Received for publication, November 11, 1999, and in revised form, February 29, 2000

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We previously reported that S-adenosylmethionine (AdoMet), a key molecule in methylation reactions and polyamine biosynthesis, enhances axenic culture of the AIDS-associated opportunistic fungal pathogen *Pneumocystis carinii*. Here we report that AdoMet is absolutely required for continuous growth. Two transporters are present, one high affinity, \( K_m = 4.5 \mu \text{m} \), and one low affinity, \( K_m = 333 \mu \text{m} \). The physiologically relevant high affinity transporter has a pH optimum of 7.5 and no related natural compounds compete for uptake. Transport is 98% inhibited at 4 °C, 24% inhibited by 20 mM sodium azide, and 95% inhibited by the combination of 20 mM sodium azide and 1 mM salicylhydroxamic acid; thus transport is active and dependent on both a cytochrome chain and an alternative oxidase. *In vitro*, AdoMet is used at a rate of \( 1.40 \times 10^7 \text{ molecules cell}^{-1} \text{ min}^{-1} \). AdoMet synthetase activity was not detected by a sensitive radiolabel incorporation assay capable of detecting 0.1% of the activity in rat liver. In addition, the AdoMet plasma concentration of rats is inversely correlated with the level of *P. carinii* in the lungs. These findings demonstrate that *P. carinii* is an AdoMet auxotroph. The uptake and metabolism of this compound are rational chemotherapeutic targets.

*Pneumocystis carinii* is a fungus that causes *P. carinii* pneumonia (PCP) in people with AIDS, patients undergoing cancer chemotherapy, and others with conditions causing severe immunosuppression. Although the frequency of PCP in HIV-infected persons has decreased dramatically over the last 8 years, due to widespread prophylaxis against PCP and more recently to the reduced immunosuppression brought on by improved anti-HIV therapy, PCP remains common in AIDS patients. Treatment for PCP is less than ideal with frequent severe side effects from the two most effective drugs, pentamidine and the combination of trimethoprim and sulfamethoxazole (cotrimoxazole, TMP-SMZ) (1). The mortality rate also remains high (21.5%) (2). Beyond the importance of *P. carinii* as a pathogen, it is also an unusual and interesting fungus for research. As a pathogen, it is also an unusual and interesting fungus for research. Unlike other fungi, *P. carinii* is not a human pathogen; it is a fungus that can only be grown axenically. Despite the universal activity of AdoMet synthetase, exogenous AdoMet can have an effect on wild type cells. For example, added AdoMet can stimulate budding and outgrowth as well as RNA and protein synthesis in *S. cerevisiae* (6). Another example is patients recovering from liver cirrhosis which results in low AdoMet synthetase activity; these patients are benefited by supplementing their diet with AdoMet (7, 8). Since AdoMet has been reported to enhance growth of another fungus (6), we added AdoMet to the medium we used to develop an axenic culture for *P. carinii*. This caused a qualitative and quantitative improvement in *P. carinii* culture including the production of cysts. Because of the positive effect of AdoMet on axenic culture of *P. carinii* culture and because analogues of AdoMet have been explored as pharmaceutical leads, we examined *P. carinii* for the possibility of an AdoMet requirement, for the ability to transport AdoMet, for the rate of use of AdoMet and for the capacity to synthesize this essential intermediate. We found that *P. carinii* has a transport system for AdoMet which seems completely AdoMet-specific. We also found that *P. carinii* is an AdoMet auxotroph, a condition not previously reported for any cell.
S-Adenosylmethionine and P. carinii

EXPERIMENTAL PROCEDURES

Chemicals—[methyl-14C]AdoMet was purchased from Moravek Biochemicals Inc. (Brea, CA). [methyl-14C]Methionine was from Amersham Life Science (Buckinghamshire, United Kingdom). Ultra-pure AdoMet was purchased from Research Biochemical International (Natick, MA). Putrescine, ferre pyrophosphate, L-cysteine, glutamine, L-methionine, ATP, hypoxanthine, adenine, di-sodium phosphate, monopotassium phosphate, sodium chloride, trisodium citrate, trisodium citrate, potassium chloride, S-adenosyl-L-homocysteine, dibutylylphthlate, acetonitrile, perchloric acid, heptane sulfonic acid, S-adenosyl-l-methionine, KCl, HEPES, MgSO4, 1,7-diaminohexaphane, and methylthiaioadenosine were from Sigma. AccQ.Fluor reagent kit was from Waters Corp. (Milford, MA).

Culture of P. carinii—In vitro axenic culture of P. carinii was as described previously (9). Briefly, P. carinii cells were maintained in medium consisting of minimum essential medium with Earle’s salts (Life Sciences, Grand Island, NY) supplemented with 20% horse serum (Life Sciences), and 80 μg ml\(^{-1}\) of each of the following: putrescine, ferre pyrophosphate, L-cysteine, and glutamine (PenCimoc). Inocula (1.5 ml) containing 3 × 10^6 cells ml\(^{-1}\) were placed in 24-mm collagen-coated, 0.4-μm membrane pore size Transwell\(^\text{\textregistered}\) inserts held in 6-well plates (Corning Costar Corp.). An additional 2.5 ml of medium was added to the wells below the inserts. The Transwell system allows changes of medium below the insert without disturbance of the cells within the inserts. The medium was changed twice daily and at each change freshly prepared 10 ml AdoMet stock solution was added to yield a final concentration of 500 μM. Cultures were incubated at 31 °C in room temperature. For counting, cells in the inserts were suspended by agitation, samples were removed and diluted 10-fold with the following buffer: 58.5 mM diosodium phosphate, 1.5 mM monopotassium phosphate, 45.5 mM NaCl, 10 mM trisodium citrate, 10 mM dithiothreitol, and 2.7 mM KCl, pH 7.4 (SPB). Citrate and diethiothreitol were included to help dissociate clumps of P. carinii thus facilitating counting. Giemsa-stained smears as described previously (10). For some cultures growth was monitored by measuring total DNA in the culture as described (9).

Rat Model of PCP—Rats were treated with antibiotics to limit other potential opportunistic infections, immunosuppressed then intratracheally inoculated with P. carinii as described previously (11). Twenty-two days after inoculation, the rats were sacrificed. Lungs and blood samples were collected, processed, and P. carinii quantitated as described previously (12). The lungs and plasma were then stored in liquid nitrogen for up to 4 years. The thawed samples were analyzed for AdoMet content using a method depending on detection of a fluorescent adduct as described below.

Transport Measurement—For uptake studies a previously published method (13) was modified. Cultured P. carinii cells were washed three times with MEM and resuspended in SPB at a density of approximately 7 × 10^7 cells ml\(^{-1}\). Each assay had a final volume of 50 μl which included 45 μl of P. carinii cell suspension and an appropriate amount of [14CH₃]AdoMet supplemented with any other test compound in 5 μl of SPB buffer. After a suitable incubation time at 37 °C, [14CH₃]AdoMet remaining in the incubation medium was separated from the cells by microfiltering through 100 μl of dibutyl phthlate and mineral oil (1:1). The supernatants were discarded and the cell pellets were collected and counted by liquid scintillation.

AdoMet Analysis—A new method based on reverse phase ion-pair chromatography was developed for quantitation of AdoMet in P. carinii culture medium. An 80-μl medium sample from a P. carinii culture or control medium, held under the same conditions but without AdoMet or serum added, was mixed with 20 μl of 10% perchloric acid. After centrifugation at 5000 × g for 5 min to remove precipitated material, the supernatants were collected for HPLC assay. An internal standard, S-adenosyl ethionine (SAE), was added to all samples to a concentration of 13.9 μM. Chromatographic separation was achieved with an octyl silanol (C₈) reverse phase column (3.9 × 150 mm, Rainin Instrument Co., Woburn, MA) using an isocratic mobile phase of 40 mM ammonium phosphate, 8 mM heptane sulfonic acid (ion pairing reagent), and 3.6% acetonitrile (pH 5.0). The injection volume was 70 μl and the flow rate was 1 ml min\(^{-1}\). Absorbance was detected by a photodiode array (Waters 996) and recorded at 1-nm intervals from 200 to 500 nm. Chromatograms were extracted at 257 nm. Absorbance data at wavelengths other than 257 nm were used for Millennium\(^\text{\textregistered}\) (Waters Corp., Milford, MA) software peak purity algorithms and for three-dimensional computer displays which were helpful for initial method development.

An alternative method was used for measuring AdoMet in rat plasma samples. AdoMet was extracted from plasma exactly as above. Precolumn derivitization was performed as described previously for polyamine analysis (14). An internal standard of 5 μl (5 μg ml\(^{-1}\) of 1,7-diaminohexaphane) and 45 μl of borate buffer (0.2 M sodium borate, 1 mM EDTA, pH 8.8) was added to 30 μl of clarified plasma. After mixing, 20 μl of AccQ.Fluor reagent was added. HPLC conditions were as described previously for polyamine analysis (14). This method was calibrated and validated by demonstrating linearity (r > 0.99) and sensitivity in the subpicomole range using purchased authentic AdoMet.

Measurement of AdoMet Synthetase Activity—Preparation of dialyzed P. carinii extract for enzymatic analysis was as described previously (15). Enzyme assay conditions were adapted from a published method (16). Enzymatically produced AdoMet was measured by HPLC with detection either by UV absorbance as described above or by measurement of radiolabeled methionine incorporated into AdoMet using a radioactivity detector with a 300-μl solid scintillant flow cell (Radiomatic Series A100, Packard Instruments, Downs Grove, IL). Rat liver extract was used as a known source of AdoMet to validate the enzyme assay. When UV detection was used, the final reaction mixture included 150 mM KCl, 2 mM dithiothreitol, 25 mM Hepes, 500 μM ATP, 5 mM MgSO₄, 1 mM methionine in a final volume of 200 μl of which 150 μl was crude dialyzed extract. Reactions were terminated by adding 40 μl of 10 N perchloric acid and placing the mixture on ice. When AdoMet was detected by radiolabel, the assay mixture was the same except that except that 400 μM [methyl-14C]methionine was used and the final volume of the reaction mixture was 100 μl of which 90 μl was the crude extract.

Protein Assay—An aliquot of cells from each experiment was retained to determine protein concentration (dotMetric assay, Geno Technology, Inc., St. Louis, MO) and the remaining cells were used immediately for various experiments.

RESULTS

Utilization of AdoMet from the Medium—Using authentic AdoMet, a novel HPLC analytical method based on UV absorbance was developed, calibrated, and validated. The assay was shown to be linear (r > 0.99) and sensitive to the picomole range (data not shown). Fig. 1 presents AdoMet decline in culture medium over 19 h with and without cells. The decline without cells is due to spontaneous degradation due to high pH (8.8–9.2) as previously reported (17). The greater decline in the presence of cells represents AdoMet utilization by P. carinii. Based on the 101 μM difference in AdoMet concentration after 8 h with 9.0 × 10^6 P. carinii cells ml\(^{-1}\) and without cells, AdoMet usage calculates to be 1.40 × 10^7 molecules cell\(^{-1}\) min\(^{-1}\).

Effect of AdoMet on Growth of P. carinii—Growth of P. carinii in culture with and without the addition of 500 μM AdoMet was followed for 27 days (Fig. 2A). After 15 days the number of cells in cultures without AdoMet began to decline and at 27 days they were only 22% of the number in cultures to which
AdoMet had been added. This experiment was repeated (Fig. 2B) and the utilization of AdoMet was followed as the cell numbers increased. At each time point in Fig. 2B, the AdoMet content of the medium was measured at the time the cells were harvested for counting; these harvests were always 12 h after the addition of fresh medium and fresh AdoMet. The AdoMet remaining in the medium declined linearly with the increase in cell number (r = 0.90). Overall the pattern of growth is similar in Fig. 2A and 2B, with the number of cells first increasing then declining in the cultures without AdoMet. Omission of AdoMet not only reduced the total number of P. carinii cells in culture but the cells were limited to small trophozoites rather than the mixture of small trophozoites, large trophozoites, and cysts seen in cultures grown with AdoMet or isolated from infected lungs (Giemsa-stained smears, data not shown). Since there was some P. carinii growth even without added AdoMet, we examined the possibility that this was dependent on AdoMet in the horse serum component of the medium. Two lots of horse serum were analyzed and the mean AdoMet content was 32 ± 2 μM. In two lots of fetal bovine serum, the mean AdoMet content was 62 ± 15 μM. Growth of P. carinii was compared in media made with 20% dialyzed horse serum (Fig. 2C) and 20% fetal calf serum (Fig. 2D), both with and without the addition of AdoMet. Dialysis reduced the AdoMet content of horse serum to less than 2 nM. After 21 days in culture, the cells grown in media without additional AdoMet contained only 2% of the number of cells in media with added AdoMet. We also followed utilization of AdoMet from the media to which this metabolite was added and the AdoMet remaining at each time point was inversely linearly correlated with the number of cells in culture (r > 0.90).

S-Adenosylmethionine Transport—To determine the extent of AdoMet uptake, P. carinii cells were incubated for different times in SPB buffer containing 33 μM [14CH3]AdoMet and AdoMet incorporated into the cells measured as described under “Experimental Procedures.” The results shown in Fig. 3 indicate that, under these conditions, AdoMet accumulates in the cells reaching a maximal concentration at 2 min. To be sure that radioactivity measured in the cells represented AdoMet uptake rather than uptake of a breakdown product, P. carinii cells incubated with labeled AdoMet for 30 s were lysed and analyzed by HPLC. The only radioactive peak was at the AdoMet retention time confirming that the radioactivity measured in the cells pellets did represent transported AdoMet. Transport of AdoMet into P. carinii was measured over a range of pH values (4.0 to 8.5) using SPB buffer. Incubations were for 120 s and the [14CH3]AdoMet concentration was 33 μM. Transport was pH dependent with a sharp optimum at pH 7.5. To determine transport kinetic constants, P. carinii cells were
incubated for 30 s in the presence of various concentrations of $[^{14} \text{CH}_3] \text{AdoMet}$. The Lineweaver-Burk plots shown in Fig. 4 indicate the presence of two transporters. For the high affinity transporter a $K_m$ value (4.45 $\text{mM}$) was calculated and the extrapolated $V_{\text{max}}$ value was 22 nmol min$^{-1}$ mg of protein$^{-1}$. For the low affinity transporter the $K_m$ is 333 $\text{mM}$ and the $V_{\text{max}}$ 476 nmol min$^{-1}$ mg of protein$^{-1}$. Further studies were done with only the high affinity transporter because AdoMet concentrations in vivo are never high enough for the low affinity transporter to be physiologically relevant. This situation is similar to S. cerevisiae which has both a high and low affinity transporter (18). To detect whether AdoMet uptake was by facilitated diffusion or by active transport, inhibitors were used to block the production of ATP which would be required for active transport. The cytochrome inhibitor sodium azide (20 mM) reduced transport by 24 ± 4% ($n = 3$), however, the combination of the alternative oxidase inhibitor salicylhydroxamic acid (1 mM) and sodium azide (20 mM) reduced AdoMet transport by 95 ± 3% ($n = 3$). When cells were held on ice, uptake was 2% of that at 37 °C. Thus AdoMet is actively transported. Specificity of this AdoMet transporter was examined by testing the ability of compounds closely related to AdoMet to compete with AdoMet transport. The compounds listed in Table I were added to cell suspensions at the same 33 $\text{mM}$ concentration as $[^{14} \text{CH}_3] \text{AdoMet}$. The data show that none of these related compounds inhibited $[^{14} \text{CH}_3] \text{AdoMet}$ uptake. Unlabeled AdoMet did compete as expected.

$\text{AdoMet Biosynthesis}$$-$The effect of exogenous AdoMet on growth of P. carinii in axenic culture and the existence of a specific AdoMet transporter suggested that P. carinii may not be able to synthesize AdoMet and thus be dependent on the host for this metabolite. For conclusive evidence, we examined P. carinii for the presence of AdoMet synthetase, the sole enzyme required for AdoMet biosynthesis from ATP and methionine. Rat liver homogenate supernatant was used as a known source of activity to validate the assay. UV detection of thionine. Rat liver homogenate supernatant was used as a

$\text{TABLE I}$

| Competition with $[^{14} \text{CH}_3] \text{AdoMet}$ transport | $[^{14} \text{CH}_3] \text{AdoMet}$ Uptake ($n = 3$) |
|----------------|------------------|
|                  | nmol/min/mg protein |
| Control          | 2.05 ± 0.26 |
| AdoMet (without label) | 0.87 ± 0.19 |
| Methionine       | 2.08 ± 0.21 |
| ATP              | 2.18 ± 0.16 |
| Adenine          | 2.14 ± 0.26 |
| Hypoxanthine     | 2.03 ± 0.18 |
| Decarboxylated AdoMet | 1.97 ± 0.15 |
| S-Adenosylhomocysteine | 1.97 ± 0.12 |
| Methylthioadenosine | 1.89 ± 0.22 |
| S-Adenosylethionine | 1.94 ± 0.13 |

cinate that 455 pmol of AdoMet would have been produced. Inspection of the chromatogram spiked with labeled AdoMet shows that this method could easily detect 0.5 pmol of AdoMet. Based on this, the AdoMet synthetase activity of P. carinii can be no more than 0.1% of that of rat liver, and we conclude that P. carinii has no AdoMet synthetase activity. We can eliminate the remote possibility that some AdoMet was produced by the P. carinii lysate but consumed by other reactions, therefore not detected. Such reactions would be either decarboxylation by AdoMet decarboxylase or loss of the radiolabeled methyl group by a methylation reaction. For the case of decarboxylation, the HPLC conditions used would have produced a peak of radioactivity at the retention time of 25 min but there was none (data not shown). Decarboxylated AdoMet cannot be metabolized further because dialysis removes the required aminopropyl acceptors. For the case of methylation reactions, small molecule methyl receptors were removed by dialysis of the extract. Large molecule methyl receptors would most likely have been precipitated by perchloric acid at the final 10% concentration.

FIG. 3. Accumulation of AdoMet by P. carinii. Uptake was measured using AdoMet at 33 $\mu$M and conditions as described under “Results.” Data points are mean (±S.D.) from three independent experiments. AdoMet accumulation is linear for 60 s.

FIG. 4. Lineweaver-Burk plot of AdoMet transport. Incubations for transport were for 30 s and AdoMet concentrations ranged from 0.1 to 290 $\mu$M. The upper regression line in panel A was calculated from data points relevant to the high affinity transporter (2–15 $\mu$M AdoMet). The lower regression line in panel A is the same as the regression line in panel B and was calculated from data points relevant to the low affinity transporter (50–300 $\mu$M AdoMet).
and a check of the precipitate revealed no radioactivity. If large molecules were not precipitated, they would have remained in the extract applied to the HPLC column but the only radioactivity peaks were accounted for by the commercially supplied radiolabeled methionine. Extensive washing of the column eluted no additional radioactivity.

AdoMet Use in Vitro—Having shown that AdoMet is required by P. carinii and is depleted from culture medium, we considered the possibility that P. carinii might cause a reduction in the plasma AdoMet concentration in infected animals. Concentrations of most plasma components are held relatively constant by a negative feedback system but since individual cells all synthesize AdoMet, we thought it possible that AdoMet could be an exception so that an increase in utilization from the plasma AdoMet concentration in the lungs. The negative relationship is striking. This result also indicates that the requirement for AdoMet is not a culture artifact.

**DISCUSSION**

AdoMet is an essential intermediate in the physiology of the cell, both for synthesis of polyamines and as a methyl donor for nearly all methylation reactions (3). To our knowledge, all cells investigated previously were found able to synthesize this intermediate from ATP and methionine using the enzyme AdoMet synthetase; AdoMet has never been described as an essential nutrient. To understand the reason underlying our observation that adding AdoMet to culture medium qualitatively promotes P. carinii growth (9) and to expand our general investigation of P. carinii polyamine metabolism (10, 15, 20, 21), we examined P. carinii both for the ability to utilize exogenous AdoMet and to synthesize this compound de novo. The results indicate that P. carinii has an absolute need for AdoMet and to date is the only cell known to be a natural AdoMet auxotroph.

The data reported here demonstrate the degree to which AdoMet both maintains and increases P. carinii growth in vitro. It is interesting to note that in the cultures with whole horse serum but without AdoMet, the initial growth followed by a slow decline is reminiscent of the growth in culture systems described prior to our recently published axenic system (9). Previous culture systems (22) utilized a mammalian feeder cell and very likely these cells provide a significant but limited amount of AdoMet. A similar role is played by the high concentration of AdoMet in horse serum relative to the more frequently used fetal calf serum. One could speculate that addition of AdoMet to a culture system using mammalian cells would allow continuous passage to fresh feeder cells. We were able to confirm AdoMet utilization by showing that P. carinii causes depletion of this metabolite from the growth medium and this is nicely supported by the in vivo data showing that P. carinii infection causes rat plasma to become depleted of AdoMet. Although our initial culture medium contained 500 μM AdoMet and this concentration was used for the studies reported here, we have found that 50 μM AdoMet is adequate to support growth. Starting at 50 μM, the spontaneous rate of AdoMet degradation in the medium predicts a decline to 14 μM or 5.6 μg ml⁻¹ of medium at 12 h, somewhat less than reported AdoMet concentration in rat lungs, 11.1 μg g⁻¹ of tissue (23). One could speculate that the now popular use of AdoMet as a nutritional supplement could result in increased vulnerability to infection with P. carinii but there is no direct data to support this.

Kinetics of AdoMet uptake indicate the presence of a transporter and the effect of metabolic inhibitors indicate that transport is an active, energy-requiring process. One of the most interesting kinetic findings was with natural compounds related to AdoMet none of which competed with AdoMet for transport. These compounds include: L-methionine and ATP (the components of AdoMet), hypoxanthine (a purine frequently scavenged by cells), adenine (the purine moiety of AdoMet), S-adenosylhomocysteine (AdoMet missing the S-methyl group), decarboxylated AdoMet (AdoMet missing the carboxyl group of methionine), methylthioadenosine (AdoMet after decarboxylation and donation of a propyl group), and S-adenosylethionine (an analogue with the S-methyl group substituted by an S-ethyl group). Therefore, it seems that the transporter recognizes neither any component of AdoMet nor any physiologically modified AdoMet suggesting that this transporter may recognize AdoMet alone. AdoMet transport by S. cerevisiae is similar to P. carinii in being facilitated by a high
affinity transporter which is pH and energy dependent with a similar $K_m$ (3.3 μM) (24). However, this S. cerevisiae transporter is not strictly AdoMet specific since both S-adenosylhomocysteine and S-adenosylmethionine can compete with AdoMet although adenosine, methionine, and homocysteine do not (24). A recent study (18) reported an additional transport system in S. cerevisiae as well as cloning the originally described transporter. The originally described transporter is coded by the SAM3 gene and shows homology to the family of amino acid transporters. The newly described transporter is of low affinity ($K_m = 160 \mu M$), operates by facilitated diffusion and is not able to supply the needs of the cell fully, even at high concentrations of AdoMet. We also detected a second transport system in P. carinii but with a $K_m = 330 \mu M$ it cannot be physiologically significant. It seems likely that the low affinity transporter activity is due to an entirely different transporter that will recognize AdoMet when it is at very high, non-physiological concentrations. The AdoMet transporter of African trypanosomes is as specific for AdoMet as the P. carinii transporter, although it operates by facilitated diffusion rather than active transport (25). In mammalian cells AdoMet penetrates poorly and extracellular AdoMet is mainly utilized to methylate phospholipids on the surface of plasma membrane (26).

The effect of metabolic inhibitors on AdoMet transport suggest that P. carinii utilizes both a cytochrome-mediated electron transport system and alternative oxidase-mediated system for energy production. Azide, which blocked transport by 24%, is a standard inhibitor of cytochrome-mediated ATP production. Salicylhydroxamic acid, which in combination with azide blocked transport by 95%, inhibits the enzyme alternative oxidase which provides a branch in electron transport at the level of ubiquinol. Although alternative oxidase itself does not lead to ATP generation by pumping protons, it does support transport of electrons to molecular oxygen, thus allowing ATP generation based on site 1 of oxidative phosphorylation as well as substrate level phosphorylation in the mitochondrion (27).

Using an oxygen electrode, we have directly observed the ability of salicylhydroxamic acid to inhibit oxygen consumption (data not shown).

We conclude that P. carinii is an AdoMet auxotroph for the following reasons. AdoMet is necessary to support growth in vitro. P. carinii causes depletion of AdoMet from the culture medium. P. carinii has an AdoMet-specific transporter and no AdoMet synthetase activity. Strong evidence that these are not in vitro artifacts is provided by the finding that the concentration of AdoMet in rat plasma declines in proportion to the degree of P. carinii infection. An excellent chemotherapeutic opportunity is presented by this dependence of P. carinii on exogenous AdoMet and the presence of a specific transporter as well as the fact that mammalian cells do not transport AdoMet well. Although normal metabolites related to AdoMet do not inhibit transport, it may be possible to design analogues that are recognized by the transporter with equal or greater affinity than AdoMet. If transported in place of AdoMet, such compounds can also be expected to interfere with AdoMet-dependent methylation and with polyanine biosynthesis. This dual mode of action would be synergistic because suppression of AdoMet transport will increase the ratio of analogue to AdoMet within P. carinii thus enhancing the effect of the analogue on AdoMet utilizing enzymes. Because mammalian cells do not actively transport AdoMet, the chances of developing a compound with high specificity of P. carinii are good. Analogues of AdoMet have already been synthesized and considered as pharmaceutical leads for other infectious agents (28); a compound useful as an anti-P. carinii agent may be among these.

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*J. Biol. Chem.* 2000, 275:14958-14963.
doi: 10.1074/jbc.275.20.14958

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